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SPIROCHÆTA (TREPONEMA) PALLIDA AND SYPHILIS.

By SIMON FLEXNER, M.D.

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PLATE XVII.

The extensive bibliography on experimental syphilis and spirochæta pallida has been brought together and reviewed recently by Hoffmann,¹ Neisser,² R. Kraus,³ Glass,⁴ and Buschke and Fischer,⁵ in whose publications the present state of our knowledge on these subjects can be readily found. The weight of opinion is strongly in favor of accepting the transmissibility of the syphilitic virus to the lower and the higher monkeys, and to the eyes of rabbits, guinea-pigs⁶ and dogs.⁷ The almost constant demonstration of spirochæta pallida in syphilitic products and in the organs of foetuses and infants inheriting active forms of syphilis, and the close association of the spirochætæ with the specific lesions, has led the majority of workers in this field to look upon the pallida as the probable cause of syphilis. The criticisms which have been made of this view have, in the main, not weakened it seriously, and must, in the nature of things, remain as criticisms until such time as the pallida is obtained in pure cultivations with which inoculation tests can be carried out. Apparently we are no nearer the cultivation of the pallida than we were immediately after its discovery. The criticism that has, in the past, aroused the largest degree of interest holds that the tissue spirals, exhibited by silver impregna-

¹ Hoffmann, Die Ätiologie der Syphilis nach dem gegenwärtigen Stand unserer Kenntnisse, Berlin, 1907.

² Neisser, Die experimentelle Syphilisforschung nach ihrem gegenwärtigen Stande, Berlin, 1906.

³ Kraus, Handbuch der Hautkrankheiten, 1905, p. 318.

⁴ Glass, Inaug.-Diss., Leipzig, 1906.

⁵ Buschke and Fisher, *Arch. f. Dermatol. u. Syphilis*, 1906, lxxxii, 63.

⁶ Bertarelli, *Cent. f. Bakt., Orig.*, 1907, xliii, 448.

⁷ Hoffmann and Brüning, *Deut. med. Woch.*, 1907, xxxiii, 553.

tion, are not micro-organisms, but histological structures such as nerve filaments, cement lines, elastic fibrils, etc. Saling and W. Schultze⁸ especially have championed this view which has found some adherents. The force of it has undoubtedly been considerably enhanced by the fact that a method for staining the pallida in tissues by means of aniline dyes has not been found, and, at the time of its promulgation, a method for silver staining of the pallida in film preparations had also not been found. It was possible to stain the pallida quite constantly in film preparations with Giemsa's stain, with Proca's stain and with many other special aniline stains; but often there was observed a striking disproportion between the large numbers of spirals of pallida form impregnated in tissues with silver and the smaller number demonstrable with anilines in the films. Differences in size and thickness of the spirals have also been noted and described, depending upon whether they were seen in film preparations stained with anilines when they appeared thin and delicate, or in tissues impregnated with silver when they appeared thicker and coarser. All these disagreements have been used to discredit the micro-organismal nature of the spirals and the identity of the film and the tissue spirals. The discussion provoked by Saling and Schultze's criticism has been wholesome in bringing out rapidly new facts relating to the pallida that would probably have come out finally, and which tend to strengthen the view of its ætiological position in syphilis. Mühlens⁹ has undertaken to give a categorical refutation of Saling and Schultze's claims.

My interest in *spirochæta pallida* began soon after its description by Schaudinn and Hoffmann and has continued until the present time. I have not worked continuously with the pallida in relation to syphilis during the intervening period, but I have taken pains to collect experience regarding the subject. Through the courtesy of the staff of the City Hospital and of Dr. Ryder of the Sloane Maternity Hospital, I have had numerous opportunities to study cases of acquired and congenital syphilis and other venereal diseases.

⁸ Saling, *Cent. f. Bakt., Orig.*, 1906, xli, 712, 812; 1906, xlii, 38, 120. Schultze, *Berl. klin. Woch.*, 1906, xliii, 1213; 1906, xliii, 1654.

⁹ Mühlens, *Cent. f. Bakt., Orig.*, 1907, xliii, 586, 674.

I have collected notes on the occurrence of the pallida in a wide variety of syphilitic lesions and on the relation of film and tissue spirals which are essentially in accord with the experiences of others who have worked with a large and varied material. The discrepancies in numbers met with, I have endeavored to remove in the case of films, by employing the Proca staining method without the alcohol fixation, with a view to enhancing the action of the mordant and increasing the certainty of the staining result. This modification is an advantage in certain instances, since by means of it more uniform staining of the pallida could be obtained than with the original method or with Giemsa's stain in any of its several ways of application. But my experience taught me that other factors beside the aniline stain, or the particular manner of its application, affected the result. Just what these factors are, I cannot say, but they affect either the pallida itself or the medium in which it lies. The conclusion I reached is that silver-impregnation often exhibits many more spirals than the aniline dyes because it is effective under conditions which interfered with the anilines' giving a differential result. I experimented somewhat with the direct silver staining of spirochætæ in film preparations without success, until Stern¹⁰ published his method which is very simple and very effective and has already removed many of the discrepancies which I had previously observed. With this introduction, I propose to record a few of my notes, since they bear upon certain contested points in the ætiology of syphilis.

Appearance and Persistence of Spirochæta pallida in Secondary Lesions.—The patient, a young woman, was exposed to venereal infection in November, 1906, and noticed the first lesions, consisting of condylomata and skin maculæ, in February, 1907. I first saw the patient early in March, at which time the condylomata had nearly disappeared, but the maculæ were distinct over the back and chest. On March second, there appeared on the lower lip, near the left angle of the mouth, an elevated flat papule of pale grey color, covered with intact epithelium. The epithelial layer was lightly scraped away with a curette and impression and smear films made from the exuded serum. The films stained by the modified Proca and by the Giemsa method gave very many typical *S. pallida*. The patient was at this time receiving deep injections of mercurial salts. The papule increased in size in the next days, and on the twelfth instant, a second examination was made with similar results. On the twentieth

¹⁰ Stern, *Berl. klin. Woch.*, 1907, xliv, 400.

instant, the papule was stationary and the examination gave many typical pallida. On April 12, this papule had begun to regress and a second papule, smaller in size, made its appearance on the lower lip near the opposite angle of the mouth. Films made from both papules on this date showed fewer pallida in the first lesion than on the previous examinations, and pallida in the second lesion but fewer in number than in the first papule. These papules were examined at intervals of one or two weeks, until they gradually disappeared. The second one to develop was never as rich as the first in pallida; and the first papule continued to show pallida in diminishing numbers until its final disappearance about June 1. During this period of examination, other papules appeared on the tongue and pharynx and they showed the pallida on examination. While these lesions were developing or remaining stationary, the skin maculæ were disappearing.

The films from the papules of the lip never showed any micro-organism except *S. pallida*, while those from the tongue and pharynx, with which the mouth secretions became admixed, showed other micro-organisms including the mouth spirals. It is worth mentioning that evidences of degeneration or dissolution were not found in the pallida obtained from the disappearing lesions of the lip. During the stationary period, the number of pallida was large and the forms distinct. The number of the pallida decreased very slowly, even when regression of the lesion set in, but the forms remained distinct. Fragmented spirals were never observed.

A large number of films were prepared at the different examinations and stored in the refrigerator at 2°-4°C. They retained their staining properties unimpaired for many months. Subsequently, some of these were stained directly with silver nitrate which brought out the spirals with great distinctness. The number exhibited was about the same as was shown by the Proca stain and depended somewhat upon the length of immersion in the silver bath. The silver is precipitated slowly upon the spirals, and hence a deeper and more complete impregnation is secured after an exposure of from three to five days than after a shorter exposure. When the impregnation is less perfect, the spirals appear broken into a series of comma-like figures, or they are indicated by a sinuous line of dots.¹¹ Longer immersion usually brings out the unbroken spirals in strong relief. The precipitation in the blood corpuscles and serum is so fine as to give merely a faintly colored ground against which the intensely black pallida stand out sharply. Disturbing coarse precipitates do not occur. Heavier films can be employed for the silver impregnation than for many of the anilines.¹²

This case is of interest in showing the close and immediate connection of *spirochæta pallida* with the developing and persisting

¹¹ Stern, *op. cit.*

¹² The length of the immersion in the silver bath will be determined by the strength of light to which the immersed films are exposed. It is, I think, better to employ very weak and diffuse light and not strong diffuse light, so as to bring the silver down slowly and to avoid deep coloration and objectionable precipitations. The direction of Stern to wait until a metallic film appears is useful, but this film often appears before the impregnation is complete. Excessive impregnation causes the spirals to appear coarser and of uneven contour.

secondary lesions of syphilis, and the gradual disappearance of the pallida, without exhibiting direct evidences of dissolution, with the regression of the lesions. The facts observed are interesting for another reason, namely, that the number of the pallida brought out on the films was about equal by the mordanting Proca stain, by the Giemsa stain (immersion for one or two days), and by the silver impregnation method.

Apparent Discrepancy in the Finding of S. pallida upon the Surface and in the Interior of Lesions.—The patient was a young woman who presented many typical flat condylomata about the vulva and anus. Film preparations from a superficial scraping of a condyloma stained by Proca's and Giemsa's method showed many spirochætæ of the pallida type, some of the refringens type and other micro-organisms. Three days after the examination, the treatment in the interim having consisted of calomel powder, a condyloma was excised. Impression and smear preparations from the interior of the lesion were stained by the methods used for the superficial scrapings, but no spiral or other micro-organisms were shown. A very careful and laborious search was made of many films without finding any pallida. After the silver impregnation had been used successfully on films from other cases, it was applied to the films, some of which had been preserved in the refrigerator, prepared from the interior of the condyloma. All the films impregnated with silver showed typical pallida type of spirals.

The second patient was a man who presented himself some weeks after the appearance of an ulcerated lesion of the glans penis. A typical skin eruption and general adenitis existed. Circumcision was performed, and when I examined the patient, the ulcer was clean and indurated. Films were made from the lymph exuded after light curetting. No spiral organisms could be found upon staining with the Proca and the Giemsa methods. Silver impregnation gave a small number of typical spirals of the pallida type.

A discrepancy has been noted repeatedly in the number of pallida shown in films and the far greater number shown in tissues impregnated successfully with silver, and certain discrepancies have also been noted in the clinical appearances, suggesting unmistakable syphilitic infection and the successful demonstration of the pallida in the lesions. It is true that with the development of greater facility in examination, fewer failures in these examinations have to be recorded; but it is also true that the number of pallida demonstrable in the films may be very small. It would appear as if the medium in which the pallida at times finds itself may affect the staining result considerably. I am inclined to regard the medium as influencing the staining in certain instances rather than a peculiar

condition of pallida itself. Apparently the unfavorable condition of medium is less effective against the silver impregnation, and hence I am disposed to think that by employing it systematically in this class of examinations fewer discrepancies will have to be recorded in the future. A very striking example of the great value of the silver impregnation of the films is supplied by an examination of a macerated foetus, the facts of which follow.

Congenital Syphilis with Colony-like Masses of S. pallida.—The mother had three previous pregnancies. The first was normal, the child dying at three months of age; the second was a miscarriage at the third month; the third child was still born. Two years ago, she had a general rash followed by pharyngitis and alopecia and ulcers of legs. Scars of the last still remain. The mother felt signs of life in her present pregnancy until two weeks before entering hospital. During the two days she was in hospital before the miscarriage, no signs were discovered. Miscarriage at about the seventh month. Moderate degree of maceration of foetus; no decomposition. The epidermis had come way over a large part of the surface of the body exposing a pinkish cutis showing punctate hæmorrhages. The peritoneal cavity contained much dark, blood-stained fluid. The organs were moderately macerated. Films were made from the skin surfaces, the lungs, liver and adrenal glands and were stained by Proca's method and impregnated with silver.

The Proca-stained films show a small number of pallida in the skin and adrenal gland; while the corresponding films impregnated with silver show large numbers of pallida. The microphotograph (Plate XVII, Fig. 1) shows a colony-like mass and outlying smaller groups of the pallida present in a silver-impregnated film from the skin magnified one thousand diameters, and the microphotograph (Plate XVII, Fig. 2) shows a number of isolated pallida of the same film magnified two thousand diameters. Evidence of transverse division is seen among the spirochætæ.

The films prepared from this foetus show clearly the great discrepancy which may occur between the results obtained by aniline staining and silver impregnation of the pallida. There can be no doubt that the masses of pallida brought out in the films by the silver-impregnation agree better with the appearances presented by silver-impregnated tissues than the smaller numbers shown by the aniline dye. That the pallida grows sometimes in large felted, colony-like masses on the cutis is proven by this observation. Whether equal growth ever takes place in the living tissues, or whether it is only in the dead foetal tissues retained in utero that such development takes place, is an open question. Doubtless foetal tissues are very favorable to the multiplication of the pallida, and

it is easily conceived that the dead, sterile tissues of the foetus maintained at body temperature, might form a suitable soil for unrestricted growth of the spirochætæ. Many of the changes taking place in the tissues of the macerated foetus are the result of autolysis; but the products of this autolysis are without active dissolving effect on the pallida and they would appear, in view of the possibility of post-mortem development of the pallida just suggested, not to restrain effectively its growth. I have evidence (see below) that the pallida is far less subject to the disintegrative influences of autolytic tissue ferments than the body cells. On the other hand, I have studied the tissues from a congenitally syphilitic child, dying on the 12th day after birth, in which such a rich development of the pallida had taken place in the lungs and liver (perhaps elsewhere also) as to recall the masses seen in the film from the skin just described. Since so much stress had been laid by some critics of the pallida in relation to syphilis upon an apparent discrepancy in the number of the pallida occurring in macerated foetuses and in syphilitic infants born alive, I shall describe briefly this instance and two or three others bearing on the latter point.

Spirochæta pallida in Congenitally Syphilitic Infants.—Case 1. Infant died on the twelfth day after birth from repeated and uncontrollable hæmorrhages from the umbilical cord and the gastro-intestinal mucosa. Autopsy performed twenty-four hours after death. The histological examination showed syphilitic pneumonia, and interstitial hepatitis, splenitis, pancreatitis and nephritis. The lung and liver were impregnated with silver nitrate by the original Levaditi method. The lung sections show countless myriads of the pallida in the interstitial tissue and in the alveoli containing the desquamated epithelium, and surrounding and penetrating within the lumen of the bronchi. Many, but fewer, pallida are present in the liver sections within and between the liver cells and in the interstitial tissue.

Case 2. Infant lived one day. Autopsy twenty-four hours after death. Anatomical diagnosis: white pneumonia; syphilitic perisplenitis, hepatitis and nephritis. Sections of the lungs and liver show many typical spirochætæ pallidæ.

Case 3.¹³ Premature infant; lived three-quarters of an hour. Mother syphilitic. Autopsy four hours post mortem. Pallida numerous in the skin, in small numbers in the liver and the bile (Proca stain).

Case 4.¹⁴ Mother contracted syphilis seven years before present pregnancy; no symptoms at present. The living child shows a roseolus and papular rash over legs, trunk, etc. A few drops of serum and blood were expressed from an incised macule of the foot; they were rich in spirochætæ.

¹³ Flexner, *Medical News*, 1905, lxxxvii, 1106.

¹⁴ *Idem*.

Case 5. Slightly macerated foetus of sixth month. Mother entered hospital in the sixth month of pregnancy on account of condylomata and oedema of vulva. Examination of a condyloma (Proca and Giemsa stain) gave many pallida. Abortion: pallida in the foetus.

The next example (Case 6) is that of a slightly macerated foetus of the eighth month showing extensive areas of white pneumonia (adhesions existed between the pneumonic areas and the chest wall). The pneumonic areas showed large numbers of the pallida, while the adjacent non-pneumonic lung tissue showed few or no pallida (Proca and Giemsa staining). Silver impregnation showed large numbers of the pallida in the infiltrated but not in the non-infiltrated lung tissue. Silver-impregnation of films prepared four months previously and kept in the refrigerator, showed many pallida. The impregnation was less heavy than with other and more recent films, and no striking disproportion in numbers of the spirochætæ existed between the aniline-stained and silver-impregnated specimens. The mother of the foetus showed no signs of active syphilis, except a small ulcer of the tongue, three or four millimeters in extent, from the depth of which *S. pallida* was obtained.

Fragments of the lung tissue were kept in the refrigerator (2° to 4.5° C.) for three months. At these temperatures, a slow autolysis without putrefaction goes forward in the tissues. At the end of one month, the tissues were much softened and disintegrated, but the pallida were little, if at all, altered in form and staining properties. At the expiration of three months, the tissues were still further softened and disorganized (no putrefaction) and no pallida could be stained. This observation bears upon the resistance displayed by the pallida to the destructive action of the autolytic ferments.

Our knowledge of the viability of the pallida outside the body is very imperfect. I am, for this reason, led to record the following instance in which the virus was able to produce infection in a macac species of monkey after the excised chancre had been kept in the refrigerator for twenty-four hours.

Viability of S. pallida and Recurrent Syphilitic Lesion in the Monkey.—A chancre of the vulva was excised December 31, 1905, under cocaine anæsthesia. Films showed a moderate number of pallida. Accidental circumstances required the tissue to be kept in the refrigerator until the next day before inoculation of a monkey could be carried out. The inoculation was made into the right eyebrow. The scarifications healed in three or four days. Twenty-four days after inoculation, a small, elevated induration appeared at the site of inoculation, which gradually increased and extended until it reached 2.5 cm. in extent. Scales and later crusts formed over the surface which on removal showed an ulcerated area. A small portion of the node was excised for examination. The his-

tology was that of experimental chancre in the monkey. A slow and gradual recession of the lesion occurred, until at the tenth week, only a small indurated area remained. After a brief quiescent period, a new growth began, which, at the end of the twelfth week equalled the original tumefaction. After a second stationary period, the growth began again to recede about the sixteenth week, and finally quite disappeared. The histology of the recurrent lesion was similar to the original.

I shall not comment further on these notes which are presented as evidence of the relationship of *spirochæta pallida* to acquired and congenital syphilis.

ADDENDUM.

Since the above paper has been in the hands of the printer, Schmorl (*Deut. med. Woch.*, 1907, XXXIII, 876) has succeeded in devising a method for the demonstration of *spirochæta pallida* in the tissues of congenitally syphilitic infants, stained by Giemsa's methods. This demonstration removes another of the objections urged against the microörganismal nature of the spirals and correlates still further the appearances observed in the films and in the tissues.

PLATE XVII.

FIG. 1. *Spirochæta pallida* impregnated with silver. Film prepared from the skin of a macerated, congenitally syphilitic fœtus. Magnification, 1,000 diameters.

FIG. 2. From the same film as Fig. 1. Magnification 2,000 diameters.

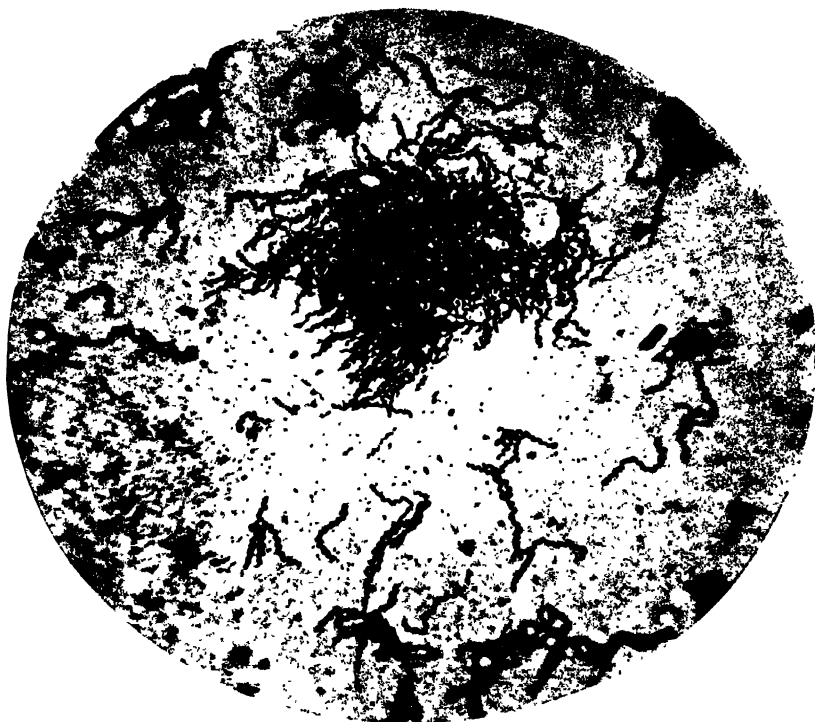
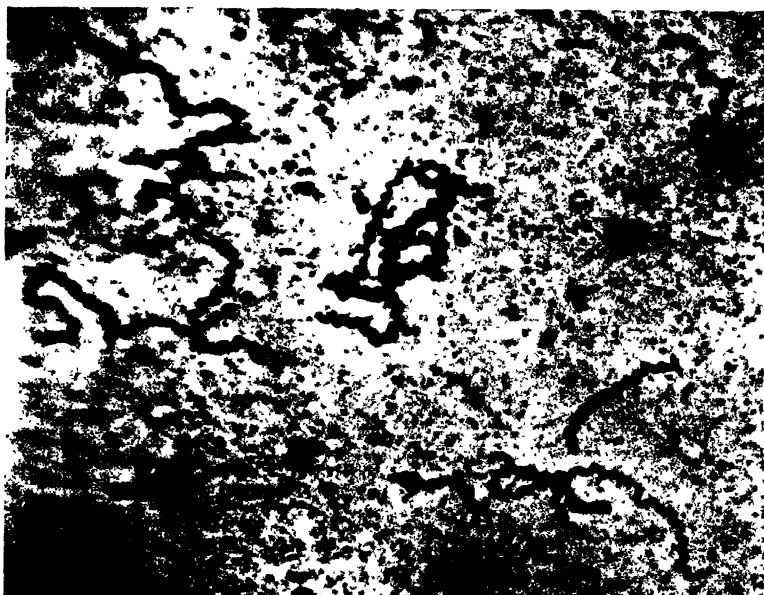


FIG. 1.



QUANTITATIVE METHODS WITH HEMOLYTIC SERUM.¹

By WILFRED H. MANWARING.

(From the Pathological Laboratory of Indiana University.)

(Received for publication, June 13, 1907.)

In a previous paper,² it was pointed out that the application of *direct* quantitative methods is at times impossible with hemolytic serum; that, for example, the direct determination of the amount of hemolytic amboceptor (specific thermostable substance) remaining in a heated³ hemolytic goat serum, after the serum has been exposed to washed sheep corpuscles, is out of the question. Work has been undertaken, during the last two years, to devise a possible *indirect* method of analysis, applicable to this problem.

Such amboceptor determinations are impossible, because heated hemolytic goat serum is so changed by contact with sheep corpuscles, that duplicate titrations do not agree. To illustrate, in one attempt, duplicate titrations gave results varying from 32 per cent to 77 per cent of the original amboceptor; in another attempt, from 70 per cent to 113 per cent, depending in each case on the volume analyzed.⁴

Heated hemolytic serum contains, in addition to amboceptor, a large number of unknown substances. These for convenience have been spoken of collectively as constituting the *third serum component*.⁵

It was found that a change in the relative amount of amboceptor and third component in a heated hemolytic serum, is sufficient to render the direct analysis of that serum impossible, by causing a non-agreement of duplicate titrations. Duplicate determinations with such altered sera gave results varying from 52.5 per cent to 64.0 per cent in one experiment, and from 5.0

¹ Work aided by the Rockefeller Institute for Medical Research.

² This *Journal*, i, pp. 213-218, 1906.

³ 55°-59° C., for 30-60 minutes.

⁴ *Journ. of Infect. Dis.*, ii, p. 490, 1905.

⁵ *Ibid.*, iii, pp. 647-662, 1906.

per cent to 9.8 per cent in another, depending on the volume analyzed; the actual amount of amboceptor present in the two experiments being 50 per cent and 5.6 per cent.¹

These disagreements, though sufficient to render quantitative work valueless, are not as marked as the disagreements observed in sera after exposure to corpuscles. Changes, other than those of the relative amount of amboceptor and third component, were, therefore, suspected.

It was found that pure third component (heated normal goat serum) suffers marked qualitative changes when exposed to sheep corpuscles. A third component originally hemolysis-increasing (auxiliary) has its auxiliary power decreased by such exposure, or even replaced by an antilytic power; while a third component originally antilytic has its antilytic power increased.²

It was further found that washed sheep corpuscles give off a powerful antilytic substance into physiological saline, and that the addition of a proper amount of this exposed salt solution to a third component, produces approximately (though not exactly) the same changes as those resulting from exposure to corpuscles.

The amount of the exposed salt solution necessary to produce this approximate change, however, is not the same with all sera. With one third component, the desired change is most closely simulated, by the addition of an equal volume of the salt solution; with a second serum, a half-volume produced the closest approximation; while, with a third sample, a double volume is required.

As a result of this work, the following changes are now conceived to take place in a heated hemolytic goat serum during exposure to washed sheep corpuscles: (i) A decrease in the amount of amboceptor, due to the absorption of amboceptor by corpuscles. This, of course, is as yet a purely hypothetical change, as an unchallengeable measurement of this decrease is at present impossible. (ii) A resulting change in the relative amount of amboceptor and third component. (iii) The giving off into the serum of antilytic corpuscles products, the amount (and possibly the nature) of which is influenced by the nature of the serum

¹ *Journ. of Infect. Dis.*, iii, p. 648, 1906.

² A detailed account of these experiments is now in press in the *Journal of Infectious Diseases*.

used. And (iv) certain minor changes, the nature of which is not understood.

These conceived changes are so complex, and each has such a marked influence on hemolytic power, that *it does not seem possible, at present, to devise an indirect method of analysis by means of which the residual amboceptor in an exposed heated hemolytic serum can be measured.*

QUALITATIVE CHANGES IN THE THIRD SERUM COMPONENT.*†

WILFRED H. MANWARING.

(From the Pathological Laboratory of Indiana University.)

IN work begun about three years ago for the purpose of testing the application of certain physico-chemical laws to hemolytic serum, a number of apparently paradoxical results were observed.¹ In attempting to determine the cause of these seeming paradoxes, the discovery was made that direct analytical methods are not applicable to many quantitative serum determinations.²

The application of quantitative methods is one of the most fundamental problems in any field of biological chemistry. It is the first problem that should be solved, before quantitative work is begun in that field. Failure to solve this problem may lead to serious error. Work was therefore undertaken to determine why the ordinary methods of analysis are not applicable to the phenomena in question, and, if possible, to find an indirect method applicable to them.

The problem immediately at hand was the measurement of the hypothetical absorption of hemolytic amboceptor by blood corpuscles. Present analytical methods are not applicable to this problem, because the heated hemolytic serum, used for the amboceptor measurements, is so changed by contact with corpuscles, that duplicate titrations of it do not agree. One of the reasons for this non-agreement is probably a change in the relative amount of amboceptor and third component in such serum as a result of the exposure³ to blood corpuscles; but the changes produced in such serum by altering

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† Presented before the Chicago Pathological Society, April, 1907, and before the American Association of Pathologists and Bacteriologists, at Washington, D. C., May 7, 1907. Work aided by the Rockefeller Institute for Medical Research.

¹ *Jour. Infect. Dis.*, 1905, 2, p. 490; *Centralbl. f. Bakt.*, 1906, 40, p. 382.

² *Jour. Infect. Dis.*, 1905, 2, p. 403; *Jour. Biolog. Chem.*, 1906, 1, p. 213; *Centralbl. f. Bakt.*, 1906, 40, p. 386; *Trans. Chic. Path. Soc.*, 1905, 6, p. 319.

³ *Jour. Infect. Dis.*, 1906, 3, p. 648.

the relative amounts of these two substances artificially, is in no case as pronounced as the changes observed in the same serum after exposure to corpuscles. It was therefore suspected that, in addition

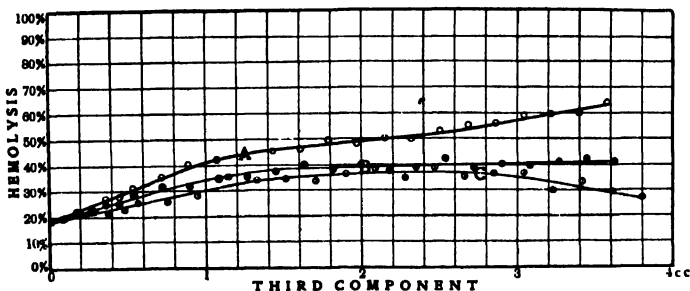


FIG. 1.—CHANGES IN THIRD COMPONENT DUE TO EXPOSURE TO CORPUSCLES.

A curve showing the changes in hemolytic power produced by increasing amounts of third component (heated normal goat serum) when added to a constant amount of hemolytic goat serum. *B* and *C*—curves showing changes produced by the same third component after contact with sheep corpuscles. The number of corpuscles used for curve *C* was greater than that for curve *B*. The constant amount of hemolytic serum used in the experiment was capable, in itself, of producing 18 per cent hemolysis. Curves *B* and *C* show a decrease in auxilic power, due to exposure to corpuscles.

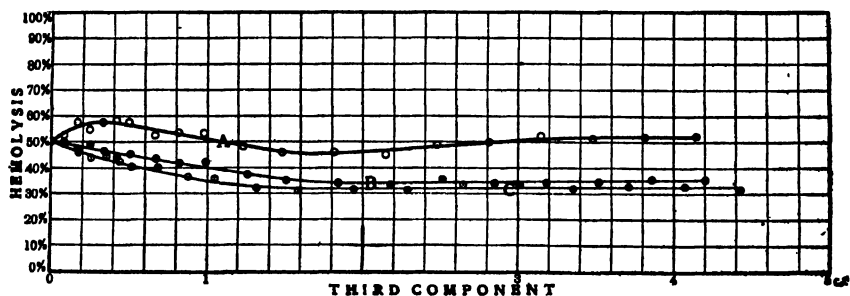
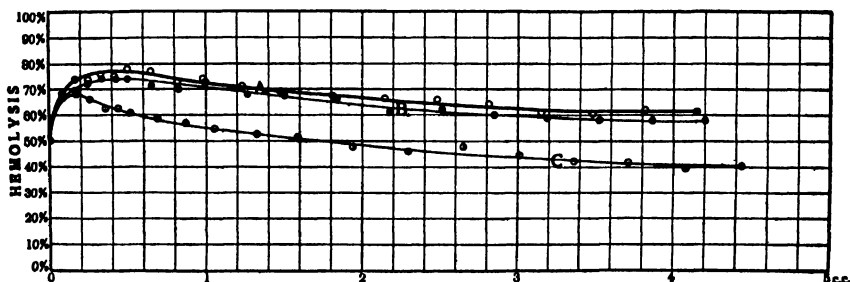


FIG. 2.—CHANGES IN THIRD COMPONENT DUE TO EXPOSURE TO CORPUSCLES.

A—unexposed third component; *B* and *C*—exposed third component. Curves otherwise similar to those of Fig. 1. Curve *C*, of the upper set, shows the auxilic power of unexposed third component replaced by an antilytic power. Curve *C* of the lower set shows a normal antilytic power increased after exposure.

to such probable quantitative changes, there are changes of a qualitative nature as well. Can such changes be demonstrated?

In order to test the existence of such possible changes, a study was made of the effect of exposing pure third component to sheep corpuscles. To do this, accurately measured quantities of third component¹ were allowed to stand in contact with carefully washed

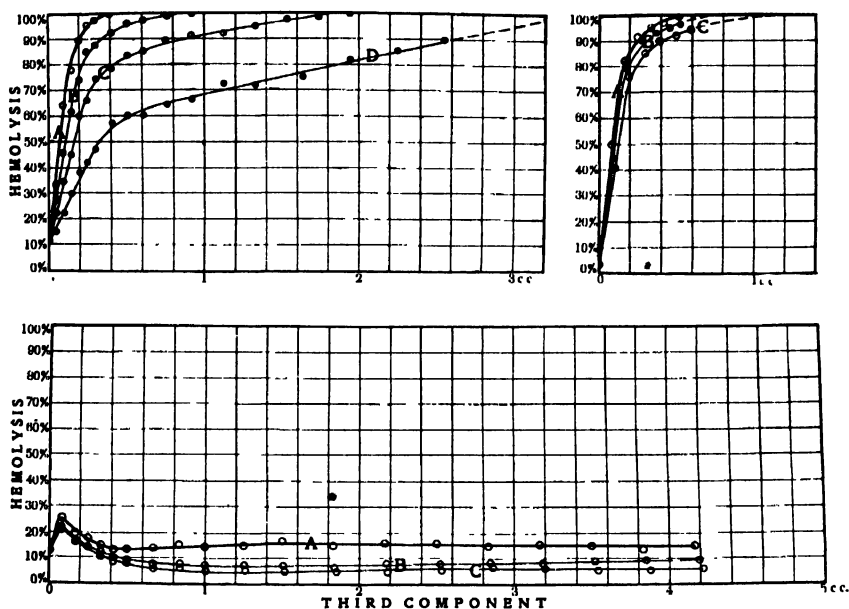


FIG. 3.—CHANGES IN THIRD COMPONENT DUE TO EXPOSURE TO CORPUSCLES.

A=unexposed third component; B, C, and D=exposed third component. Curves otherwise similar to those of Figs. 1 and 2.

corpuscles, under the same conditions and for the same length of time as in the original amboceptor absorption experiments.² The third component was then freed from corpuscles by centrifugation, and its effect on hemolysis compared with that of the original component,³ kept under identical conditions, except for the contact with corpuscles. Data, so obtained, are shown graphically in Figs. 1, 2, 3, and 4.

From these figures it is seen that exposure to corpuscles in all cases produces changes in the third component; that in every instance a

¹ Heated normal serum, containing neither amboceptor nor complement.

² For material and technic, see *Jour. Infect. Dis.*, 1905, 2, p. 461.

³ For the action of the third component, see *Jour. Infect. Dis.*, 1906, 3, p. 647.

third component that is originally auxilytic (hemolysis-increasing) has its auxilytic power decreased by such exposure, or even replaced by an antilytic power; and that a third component that is originally antilytic has its antilytic powers increased.

Three hypotheses can be put forward to account for these changes: First, that certain accessory hemolytic substances are absorbed from

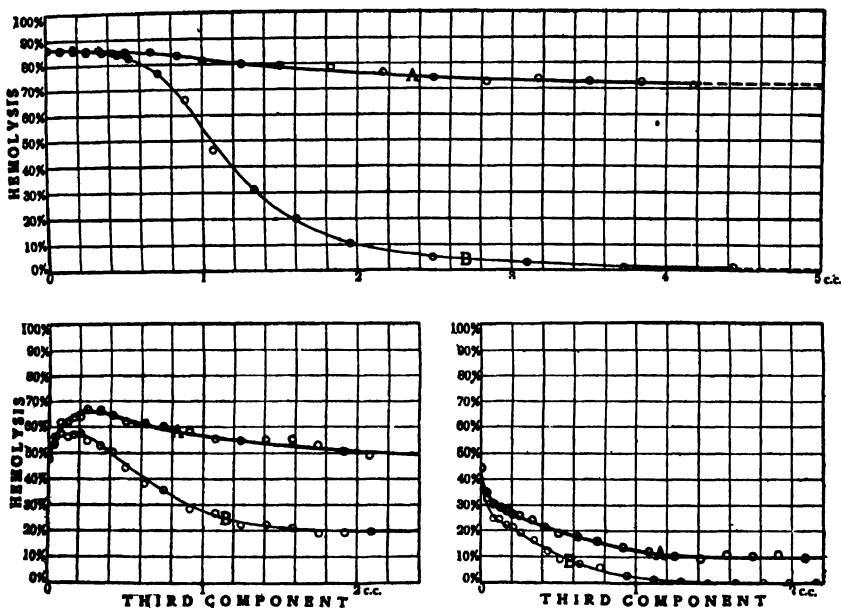


FIG. 4.—CHANGES IN THIRD COMPONENT DUE TO EXPOSURE TO CORPUSCLES.

A—unexposed third component; B—exposed third component. Curves otherwise similar to those of Figs. 1, 2, and 3.

the third component, by the corpuscles, during such exposure. Second, that certain accessory hemolytic substances are given off into the third component, from the corpuscles, during the exposure. And, third, that the corpuscles produce, independent of such absorption or giving off of products, chemical changes in the third component, presumably by the action of the enzymes they contain. Attempt was made to test these three hypotheses.

In order to determine whether or not accessory substances are absorbed by the corpuscles from the third component, carefully washed corpuscles were exposed to third component, under conditions

identical with those of the amboceptor absorption experiments above, were then freed from the third component by centrifugation, repeatedly washed in salt solution, and their susceptibility to hemolysis compared with that of unexposed corpuscles, prepared, kept, and washed under identical conditions, except for the contact with third component.

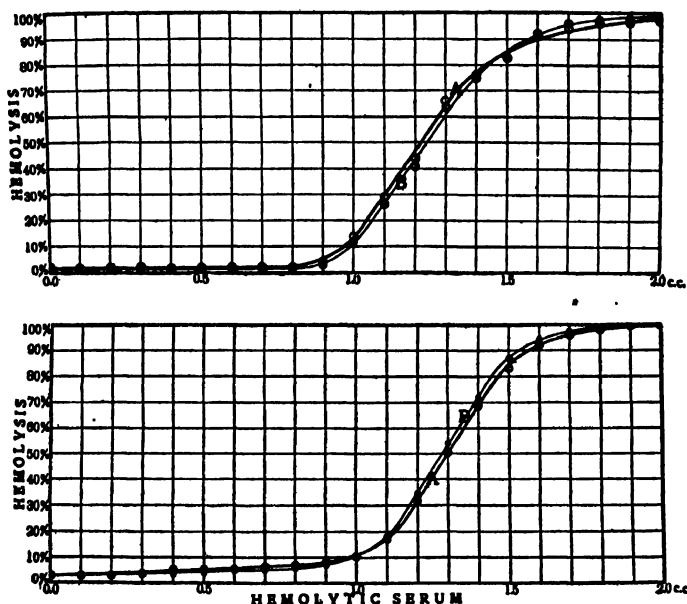


FIG. 5.—HEMOLYTIC SUSCEPTIBILITY OF CORPUSCLES AFTER EXPOSURE TO THIRD COMPONENT.

Each curve shows the percentages of hemoglobin liberated from a constant number of corpuscles, by increasing amounts of hemolytic serum. *A*—curves with unexposed corpuscles; *B*—curves with corpuscles after exposure to third component. The *B* curves show no change in hemolytic susceptibility after such exposure, within the limits of the experimental error. In the upper experiment the corpuscles were exposed to a powerfully auxilytic third component; in the lower experiment, to a third component practically inert.

The data from four such comparisons are given graphically in Figs. 5 and 6.

In the first two comparisons (Fig. 5), the corpuscles were found unchanged in hemolytic susceptibility, after exposure to third component, within the limits of the experimental error. In the second two comparisons (Fig. 6), slight changes were observed in hemolytic susceptibility, but in each case these changes were the opposite of those that would have followed a retention of the third component used.

We are therefore obliged to conclude that the hemolytically active substances of the third component are either not absorbed by corpuscles, or, if absorbed, are held in such loose chemical combination that

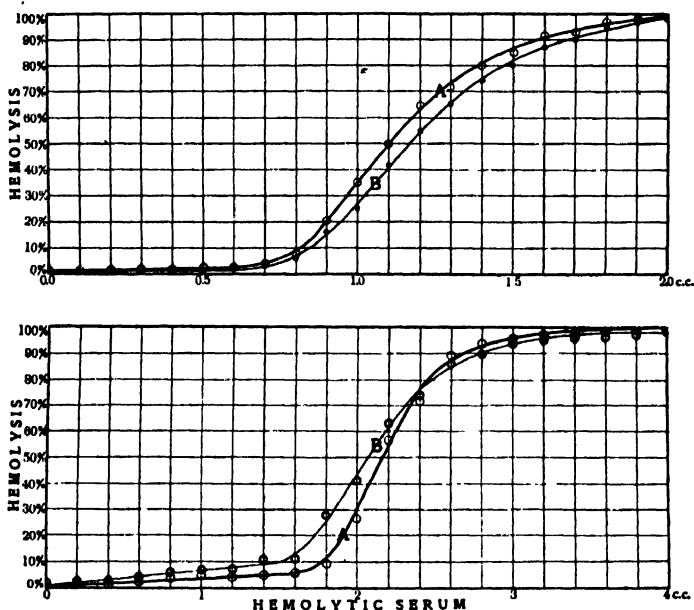


FIG. 6.—HEMOLYTIC SUSCEPTIBILITY OF CORPUSCLES AFTER EXPOSURE TO THIRD COMPONENT.

Curves, as in Fig. 5, showing changes in hemolytic susceptibility, after exposure to third component, that are not accounted for by the experimental error. In the upper experiment, the corpuscles were exposed to an auxilytic third component; in the lower experiment, to an antilytic third component. In each case the slight observed changes are the opposite of those that would have been expected from an absorption of the third component in question. The meaning of these changes is not clear, but they evidently are not changes due to third component absorption.

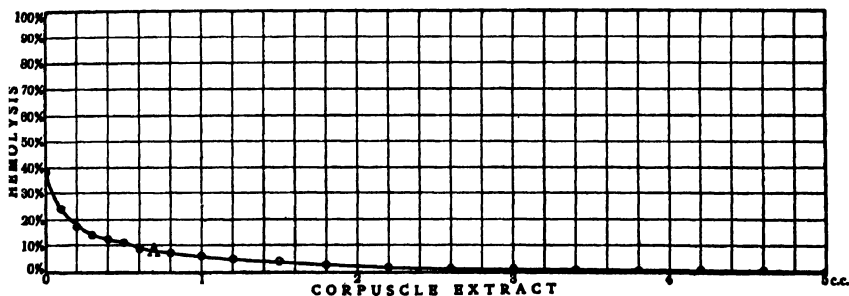


FIG. 7.—ANTILYTIC ACTION OF CORPUSCLE EXTRACT.

Curve showing changes in hemolytic power produced by adding increasing amounts of corpuscle extract (exposed salt solution) to a constant amount of hemolytic serum. The constant hemolytic serum used in this experiment was capable, in itself, of producing 38 per cent hemolysis.

they are completely removed by subsequent washings in salt solution. Absorption is experimentally undemonstrable.

In order to test the second hypothesis, that hemolytically active substances are given off from the corpuscles into the third component, washed corpuscles were exposed to physiological saline (0.85 per cent NaCl), under conditions identical with those of the amboceptor

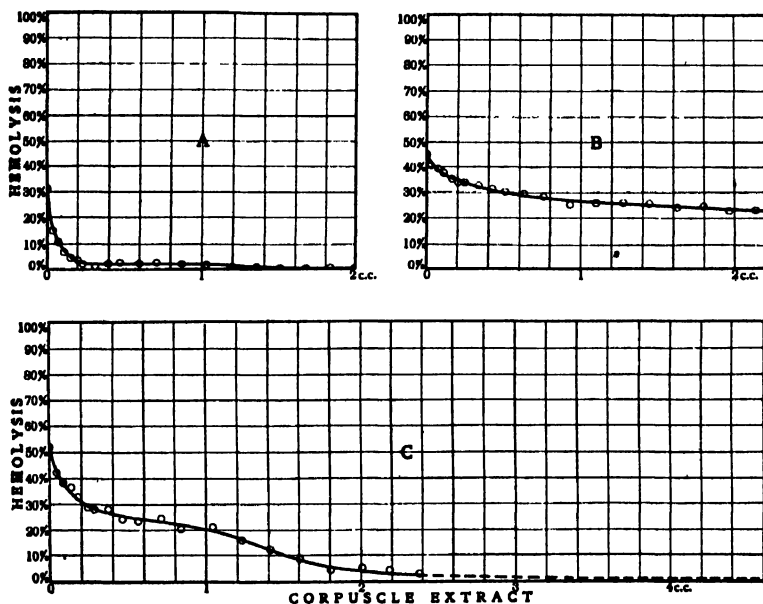


FIG. 8.—ANTILYTIC ACTION OF CORPUSCLE EXTRACT.

Curves, as in Fig. 7, showing changes in hemolytic power produced by adding increasing amounts of three different corpuscle extracts to different constant amounts of hemolytic serum.

absorption experiments above, the salt solution simply taking the place of the heated serum. The salt solution was then freed from corpuscles by centrifugation, and its effect on hemolysis tested.

It was found that this exposed salt solution (corpuscle extract) was in all cases powerfully antilytic. Such antilytic effects are shown graphically in Figs. 7, 8, and 9.

Corpuscles, therefore, give off a hemolytically active substance into salt solution. If this substance is also given off into third component, and if the giving off of this substance is the only change brought about by corpuscles in this component, it should

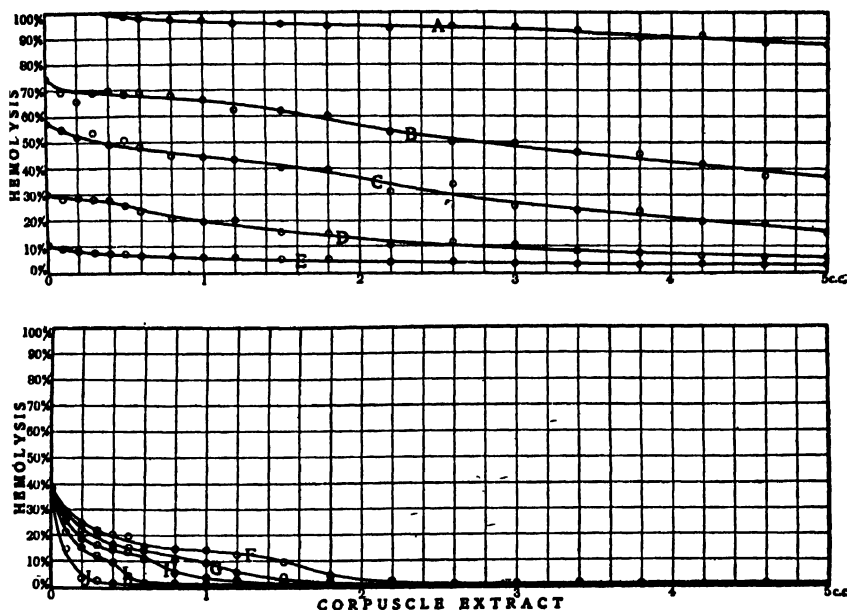


FIG. 9.—ANTILYTIC ACTION OF CORPUSCLE EXTRACT.

Curves, as in Figs. 7 and 8, showing changes in hemolytic power produced by adding increasing amounts of corpuscle extract, to constant amounts of hemolytic serum. *A, B, C, D, and E*—curves obtained with the same extract added to different constant amounts of the same hemolytic serum. *F, G, H, I, and J*—curves obtained with extracts from different numbers of the same corpuscles, added to a constant amount of the same hemolytic serum.

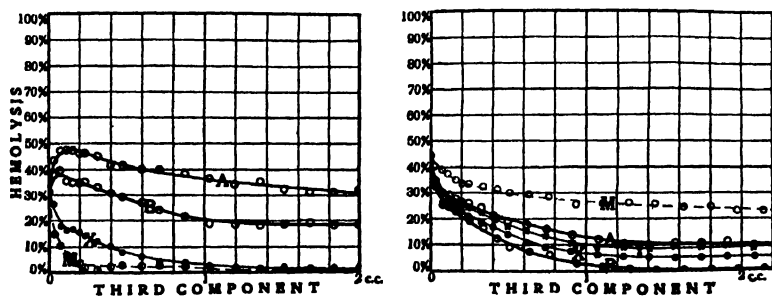


FIG. 10.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

Two sets of curves showing different effects on third component, of the addition of corpuscle extract. *A*—curve obtained with unexposed third component. *B*—curves with exposed third component. *M*—curves with corpuscle extract. This extract was in both cases obtained under the same experimental conditions, as those used in obtaining the exposed third component, salt solution simply taking place of the heated normal serum. *X*—curves obtained with a mixture of equal volumes of corpuscle extract (*M*) and unexposed third components. *Y*—curve obtained by adding a half-volume of corpuscle extract (*M*) to the unexposed third component.

In the first set of curves the addition of an equal volume of corpuscle extract produced a more marked change in the third component than the change produced in the same serum by exposure to corpuscles. In the second set of curves, the addition of an equal volume of extract produced a less marked change than such exposure.

be possible to produce artificially the observed changes, by simply adding corpuscle extract to unexposed third component. To test this possibility, various mixtures of unexposed third component and corpuscle extract were made, and the action of these mixtures compared with that of exposed third component. Four sets of data thus obtained are shown in Figs. 10, 11, and 12.

The essential curves from these figures have been grouped together in Fig. 13. From this figure it is seen that, in two of

the experiments (I and III), the addition of an equal volume of corpuscle extract to unexposed third component, conferred on the component greater antilytic powers than that acquired by exposure to corpuscles. In a third experiment (II), the addition of an equal volume conferred less auxilic powers; while in a fourth experiment (IV), it gave greater antilytic power when the component was tested in certain amounts, but less antilytic power when tested in other quantities.

The nearest artificial approximation to an exposed third component was obtained, in one of

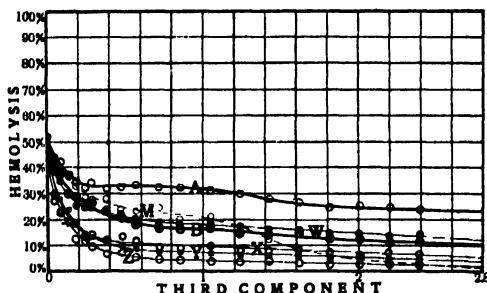


FIG. 11.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

Curves, as in Fig. 10, showing the effect on the third component of the addition of corpuscle extract. *A*—curve with unexposed third component. *B*—curve with exposed third component. *M*—curve with corpuscle extract. *W*—curve obtained by adding a half-volume of corpuscle extract (*M*) to unexposed third component. *X*—curve with the addition to an equal volume of extract. *Y*—curve with one and a half volumes of extract. *Z*—curve with twice the volume of extract. In this experiment the nearest approach to the exposed third-component curve was produced by the addition of a half-volume of extract (see Fig. 14).

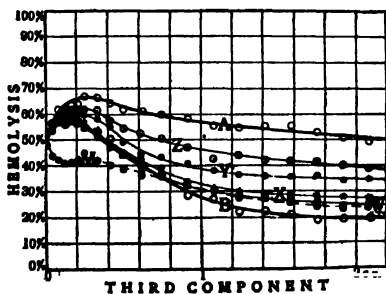


FIG. 12.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

Curves, as in Figs. 10 and 11, showing the effect on the third component of the addition of corpuscle extract. *A*, *B*, and *M*—curves with unexposed third component, exposed third component, and corpuscle extract, as before. *Z*—curve obtained by the addition of a quarter-volume of corpuscle extract. *Y*—curve with a half-volume. *X*—curve with a three-quarter volume. *W*—curve with an equal volume. In this experiment, the nearest approach to the exposed serum is made by the addition of an equal volume of corpuscle extract.

the experiments (III), by adding a half-volume of corpuscle extract. The nearly coincident curves from this experiment are shown in Fig. 14.

The addition of corpuscle extract to third component, therefore, produces changes in the third component, approximating those produced by exposure to corpuscles, but not identical with them. If we

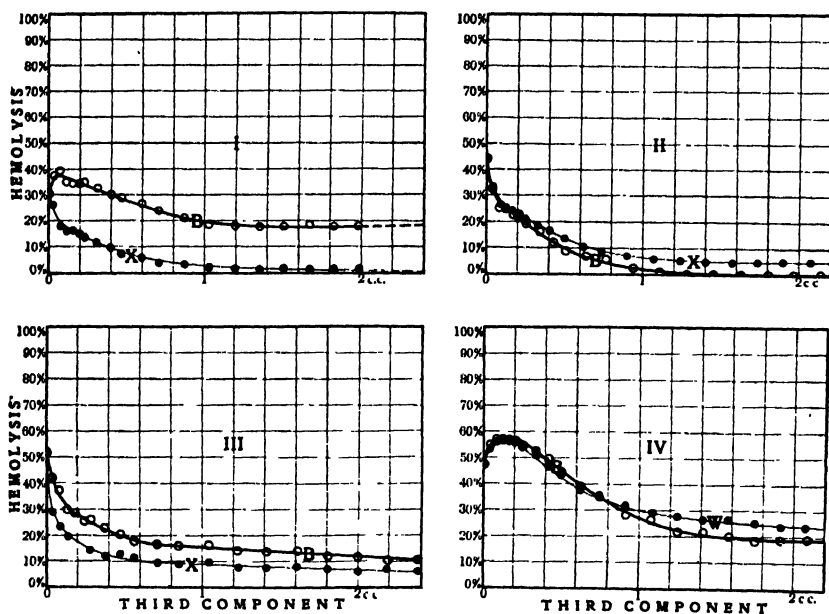


FIG. 13.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

Curves obtained with the addition of equal volumes of corpuscle extract, brought together from FIGS. 10, 11, and 12, above.

assume that the changes produced in exposed third component are mainly due to the giving off of corpuscle products into that component, it will be necessary to assume that the amount of these products so given off is influenced by the nature of the third component used; that with one third component there is given off an amount equal to that given off by the same corpuscles into physiological saline; that with another third component a much smaller amount is given off than in the corresponding experiment with physiological salt solution; while with a third serum a much larger amount is given off.

Moreover, this hypothesis would still leave unaccounted-for certain

minor changes in the third component, indicated by the crossing of the approximating curves. What these changes are, and whether or not they can be accounted for by a hypothetical digestion of third component by corpuscle enzymes, is still a matter of pure conjecture.

The qualitative changes produced in the third serum component by exposure to corpuscles are therefore such that they can neither be predicted nor reproduced artificially with sufficient accuracy for analytical purposes. This being the case, it does not seem possible at present to devise an indirect method of analysis by means of which the absorption of hemolytic amboceptor by blood corpuscles can be determined.

In the light of this conclusion, it would seem desirable to examine with care fundamental analytical data in other fields of serum pathology, to determine whether or not they rest on a sufficiently reliable foundation for the theoretical deductions now made from them.

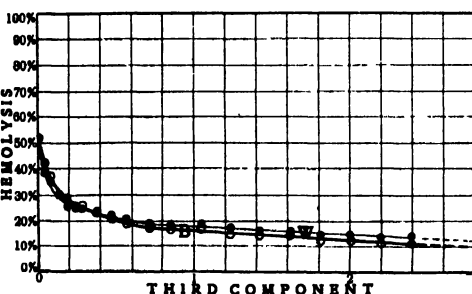


FIG. 14.—EFFECT OF CORPUSCLE EXTRACT ON THIRD COMPONENT.

The nearly coincident curves of Fig. 11.

SUMMARY.

1. Heated normal goat serum (pure third serum component) is altered in its chemical nature by exposure to washed sheep corpuscles. A third component that is originally auxilytic has its auxilytic powers decreased by such exposure, while a third component originally antilytic has its antilytic powers increased.
2. Absorption of the third serum component by the exposed sheep corpuscles is not demonstrable.
3. Washed sheep corpuscles give off into exposed physiological saline a powerful antilytic substance.
4. The addition of this antilytic corpuscle extract to unexposed third component produces approximately the same changes in the third component as those produced by exposure to corpuscles. The

amount of extract necessary to produce the desired change, however, varies with different sera. With one third component it may be the same amount as that given off by duplicate corpuscles, under identical experimental conditions, into physiological saline. With another third component, it may be a much smaller amount, and with a third serum, a much larger amount.

5. If we assume that the changes taking place in exposed third component are mainly due to the addition of corpuscle products, it will be necessary to assume certain factors in the third component that influence the amount of these products so acquired. In the presence of one third component, the giving off of these products by the corpuscles must be assumed to be stimulated; with another third component, retarded; while with a third it is not influenced.

6. Moreover, distinct though slight differences between experimental curves indicate, that, in addition to the giving off of corpuscle products into third component, there must be assumed certain minor secondary changes in the third component, the nature of which is still a matter of conjecture.

7. The changes in exposed third component are so complex, that an indirect method of determining the amboceptor absorption by sheep corpuscles seems at present impossible.

FACTORS IN HEMOLYSIS.*†

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(From the Pathological Laboratories of Indiana University and the University of Chicago.)

DURING an investigation in hemolysis, extending over about three years, a number of unsuspected phenomena have been encountered. The discovery of these phenomena has modified the comparatively simple concept of hemolytic serum held at the beginning of the work, so that in place of the two components at first regarded as the only active substances in such serum, at least a dozen components are now recognized. Some of these components have been found to be beyond experimental control, and unrecognized, to introduce serious error in hemolytic investigation.

The experimental work, of which this paper is a final summary, was done with goat serum immunized against sheep corpuscles. The substances herein enumerated, therefore, must be regarded as found only in such serum. These substances are:

1. AMBOCEPTOR. A specific, thermostable substance, or group of substances, formed in goat serum in response to repeated injections of washed sheep corpuscles. This substance is not destroyed by heating to 60° C. for several hours, or by standing at ordinary temperatures for months. Amboceptor is a necessary factor for the hemolysis, hemolysis not taking place in its absence, regardless of the number or the amount of the other serum components present.

The action of amboceptor is not understood. The current belief that it is absorbed by corpuscles rests on doubtful experimental grounds, as the quantitative analysis of an amboceptor containing goat serum that has once been in contact with sheep corpuscles is at present out of the question.¹ One can only say that contact with amboceptor in some way renders corpuscles susceptible to hemolysis

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† Presented before the American Association of Pathologists and Bacteriologists, at Washington, D.C., May 7, 1907. Work aided by the Rockefeller Institute for Medical Research. Reprinted from the JOURNAL OF INFECTIOUS DISEASES, 1908, Vol. V, p. 67.

¹Jour. Infect. Dis., 1907 4, p. 219.

by certain other serum components, which to unexposed corpuscles are non-hemolytic. No trace of amboceptor is demonstrable in normal goat serum.

2. **COMPLEMENT.** A non-specific, thermolabile substance, or group of substances, found in normal goat serum. Whether, or not the amount or nature of the complement is changed, as a result of repeated injections of sheep corpuscles, is not known. Goat complement is apparently completely destroyed by heating to 50° C. for 60 minutes, to 55° C. for 15 minutes, or to 60° C. for 5 minutes.

Complement is a necessary factor for the hemolysis, hemolysis not taking place in its absence, regardless of the number and amount of the other serum components present. In itself complement is non-hemolytic, requiring for its action the presence of a certain amount of amboceptor. Sheep corpuscles, however, that have been exposed to goat amboceptor are readily hemolyzed by complement alone. The nature and the method of action of complement are not understood.

3. **THIRD COMPONENT.** A collective name applied to the other constituents of goat serum. The nature of these constituents is impossible of experimental study in unheated sera, due to the presence of the hemolytically more active complement. A study of normal goat serum in which the complement has been destroyed by heat, however, shows that the third component then apparently consists of at least three accessory hemolytic substances, or groups of substances. These are: (1) an antilysin, or hemolysis-inhibiting substance, and (2) two auxilysins, or hemolysis-increasing substances.

The nature of these substances is not understood. Whether they are independent substances or not, and whether or not they are derivatives of complement, that is, "complementoids," have not been determined. The amounts of these substances apparently differ considerably in sera from different goats, and in the serum of the same goat at different times and under different experimental conditions. Their chemical composition, however, is apparently the same in different sera.

The third component in itself is non-hemolytic, and is incapable

of producing hemolysis in the presence of pure amboceptor or pure complement. It is believed that many current errors in hemolytic theory are due to failure to take into account the presence of, and variations in, this component.

4. **CORPUSCLE EXTRACT.** Washed sheep corpuscles probably give off into the surrounding medium at least three substances capable of influencing hemolysis. These are: (1) traces of sheep complement, whose action is apparently identical with that of goat complement; (2) traces of sheep third component, whose action is believed to be similar to that of goat third component; and (3) certain unknown substances believed to be products of corpuscle autolysis.

The principal active component in these autolytic products is a powerful, antilytic, thermostable substance, whose nature is not understood. It is the presence of this substance that renders the quantitative analysis of amboceptor-containing goat serum impossible, after the serum has been exposed to sheep corpuscles. Whether or not similar substances are given off into goat serum from its own corpuscles, has not been determined.

5. **HEMAGGLUTININ.** A specific, thermostable substance, or group of substances, formed in goat serum in response to repeated injections of sheep corpuscles. The amount of this substance varies greatly in different sera. One serum may produce complete hemagglutination in a few minutes, while another serum of apparently equal hemolytic power may be practically without agglutinating action.

The nature of this hemagglutinin is not understood. Whether or not it bears any chemical relation to amboceptor, is unknown. Its effect on hemolysis is assumed to be to decrease lysis, by clumping corpuscles and thus removing them from the action of amboceptor and complement. No trace of hemagglutinin is found in normal goat serum.

6. **ACCESSORY HEMAGGLUTININS.** At least two substances, or groups of substances, have been encountered that modify the rapidity or the completeness of the agglutinating process. These are: (1) an auxagglutinin, or agglutination-increasing substance in the third component, and (2) an antiagglutinin, or agglutination-decreasing substance in corpuscle extract. The nature of these substances is not

understood, and their relation to the other active and accessory substances in third component and corpuscle extract is not known.

7. **CORPUSCLE SUSCEPTIBILITY.** Changes in the susceptibility of washed sheep corpuscles, both to hemolysis and to hemagglutination, have been observed. The nature of these changes is not understood, and their importance in hemolytic work is undetermined. It is possible, however, that they introduce error in certain hemolytic investigations.

8. **INORGANIC SALTS, SPECIFIC GRAVITY, REACTION OF MEDIUM.** Certain inorganic constituents of serum, especially the calcium salts, are known to be powerfully antilytic. The influence of specific gravity on hemolysis is not accurately known, but it is possible that errors may arise from variations in it. The reaction of the medium, that is, its degree of acidity or alkalinity, is known to influence greatly its hemolytic action. The importance of these three factors, however, in hemolytic investigation, has not been adequately determined.

9. **HEMOPSONIN.** It is suggested by Dr. Hektoen that the corpuscles in a hemolytic tube might possibly become opsonized by the hemopsonins of the immune goat serum, and that some of them might be taken up by the leucocytes of the corpuscle suspension and thus be protected from hemolysis. Numerous smears made from routine hemolytic tubes, however, show no sign of such phagocytosis.

In the routine technique, the corpuscles are 24 hours old. The above examination, therefore, does not exclude the possibility of phagocytosis playing a rôle in hemolytic experiments under a technique involving the use of fresher corpuscles. Such a rôle, however, must be practically negligible, as it is inconceivable that the leucocytes could take up more than a maximum of $\frac{1}{4}$ per cent of the red corpuscles present, which would introduce a maximum error in the hemoglobin liberation less than the minimum difference in tint that can usually be detected by colorimetric methods.

10. **PRECIPITINS.** It is further conceivable that specific precipitins may have an influence in certain hemolytic experiments. No trace of such precipitins, however, has been found in goat serum immunized either against washed sheep corpuscles or against normal sheep serum.

SUMMARY

The substances above enumerated may be tabulated, as follows:

- | | | |
|------------------------|---|---|
| I. NECESSARY FACTORS | { | 1. Amboceptor |
| | | 2. Complement |
| II. ACCESSORY FACTORS | { | 3. Third Component |
| | | { (1) Antilysin
(2) Auxilysin I
(3) Auxilysin II |
| | | 4. Corpuscle Extract |
| | | { (1) Complement
(2) Third Component
(3) Autolytic Products |
| | | 5. Hemagglutinin |
| | | 6. Accessory Hem-
agglutinins |
| | | { (1) Serum Auxagglutinin
(2) Corpuscle Antiagglutinin |
| | | 7. Corpuscle Susceptibility |
| | | 8. Inorganic Salts |
| | | 9. Specific Gravity of Medium |
| 10. Reaction of Medium | | |

In enumerating these components, no claim is made that they are all independent substances. Nothing is known of their chemical composition, nor of the relations existing between them. Their enumeration, however, is believed to be of value, as it indicates the kind of complexities that may be expected in other fields of serum pathology. In closing this summary I wish to express my thanks to Dr. Hektoen, at whose suggestion the investigation was begun, for his generous encouragement and help throughout the work.

FURTHER STUDIES ON PUTREFACTION.¹

By LEO F. RETTGER.

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(Received for publication, October 25, 1907.)

In the present paper the writer wishes particularly to emphasize a number of points brought out in a previous publication,² and to record certain observations which have recently been made regarding the presence of putrefactive anaerobes³ in the human intestine.

Although Bienstock's contention³ that obligate anaerobes alone can bring about putrefactive changes in native proteids met with much opposition, his observations are today pretty well substantiated. It is true that here and there an investigator still claims that he has demonstrated this property in certain aërobes or facultative anaerobes, but there is lacking the evidence, either that the transformation was strictly one of putrefaction, or that the organism in question was not mixed with some contaminating anaerobe.

Fischer⁴ obtained an organism from a case of malignant stomatitis (*Bacterium stomato-fætidum*) which according to him is a putrefactive organism, and yet an aërobe. This bacterium resembles the diphtheria bacillus in morphology; it is motile, Gram-negative, and does not form spores. It grows well on all ordinary media and causes abundant gas production in dextrose and saccharose, but not in lactose. Although Fischer calls it an aërobe, he states that *some* growth takes place in an atmosphere of hydrogen or carbon dioxide. A number of different proteids were decomposed with the formation of putrefactive products.

¹ The work on which this paper is based was aided by an appropriation from the Rockefeller Institute for Medical Research.

² Rettger: This *Journal*, ii, p. 71, 1906.

³ Bienstock: *Arch. f. Hyg.*, xxxvi, p. 335, 1899; *ibid.*, xxxix, p. 390, 1901.

⁴ Fischer: *Zeitschr. f. Hyg.*, xlix, p. 329.

The statement is made that examinations by the vacuum method for contaminating anaerobes proved negative.

Should the above observations be founded on actual facts, then exceptions must be made to the proposed rule that anaerobes alone can cause putrefaction. The possibility, however, of the above organism being mixed with one or another of the so-called "ubiquitous" anaerobes can not help but impress itself on the reader.

I have within the last two years repeatedly examined at least sixty aerobes and facultative aerobes or anaerobes for putrefactive products, and in every instance obtained strictly negative results. The tests were made under different conditions of temperature, reaction, etc. When grown in unquestionably pure culture, there was no reduction in the bulk of the proteids employed, nor were there any other signs of decomposition. When mixed with one or more of the putrefactive anaerobes, the mooted transformation occurred, even when the cultural conditions did not approach those of complete anaerobiosis.

No one can deny that much of the decomposition of albuminous matter in nature is carried on by the obligate aerobes (*B. subtilis*, *B. mycoides*, etc.), but such transformation is one of ordinary dissolution or digestion. Free oxygen is always needed in abundance. This can easily be seen in ordinary test tube experiments. If we place cubes of coagulated albumin (egg or serum) in the depths of tubes containing bouillon or some other not unfavorable liquid medium, very little or no dissolution of the proteid will be observed until enough of the liquid has been removed by evaporation to expose the surface of the albumin to direct contact with the air.

Decomposition of albumin by the aerobes is never accompanied by the foul odors which are so characteristic of putrefaction. By "putrefaction" is meant here, as elsewhere in the paper, what is called "Fäulniss" in the German language.

I have never tested the group of thermophile bacteria as to their ability to cause putrefaction, and whether or not Bienstock's rule that putrefaction is limited to obligate anaerobes applies here must be further investigated. Quite recently Bardou¹ made a study of the thermophile bacteria which he

¹ Bardou: Thesis, Ref. *Centrabl. f. Bakt. Parasitenk.*, etc., i, Referate, xxxix, p. 744. 1907.

regularly found in the septic tank of the sewage purification works at Lille. Among those isolated, four appeared to be of much importance in that they had a strong digestive action on certain native proteids (egg and serum albumin, fibrin, and vegetable albumin) when kept under anaërobic conditions. It is not stated in the abstract whether this decomposition is one of putrefaction or merely of the ordinary digestive or tryptic sort; neither is it stated whether the organisms are obligate or facultative anaërobes. Reference to the original paper only will determine these points.

In summing up the more important data on this subject, one conclusion is paramount, namely; real putrefaction is the work of obligate anaërobes. If certain exceptions do occur, which has not been demonstrated with any degree of certainty, they are indeed very few, and may be safely disregarded in a general consideration of this subject.

Not all obligate anaërobes have this property of producing putrefaction. The strict anaërobes which have come under my observation may be divided into four classes, in so far as their biochemical characters are concerned. First, those that produce very little or no putrefactive change or fermentation with evolution of gas. Perhaps the best example of this class is the tetanus bacillus. Second, those that have a strong putrefactive action on native proteids but fail in fermentative properties, as illustrated by *B. putrificus* of Bienstock. Third, those which are primarily fermentative organisms, and whose putrefactive functions¹ are very slight or perhaps absent; example, *B. aërogenes capsulatus* of Welch or *B. enteritidis sporogenes* of Klein. And fourth, those which have very marked putrefactive and fermentative properties, as shown best in the bacillus of malignant edema and the bacillus of symptomatic anthrax.

It seems strange that the tetanus bacillus which is so commonly associated in nature with decaying organic matter should not possess the ability to attack native proteids, at least in a significant manner. Numerous strains of this organism have been tested by me within the past two years, relative to this point,

¹ By these terms I mean again the ability to decompose native proteids with the formation or liberation of foul-smelling products—mercaptan, aromatic oxy-acids, etc.

but in not a single instance was there any apparent action on the egg-meat mixture or plain coagulated egg albumin. It might be argued that the organisms examined may have possessed this function at one time, but that they had since lost it in consequence of the newer conditions to which they had been subjected. This may be true in a number of instances, but most of the strains tested were those that were isolated quite recently from soil, barnyard dirt, etc., and in every way appeared to be true tetanus bacilli.

Bacillus putrificus has a very vigorous action on native proteids. The intensity of this action may vary, however, depending on the age of the strain and the conditions under which it is kept for any length of time. The original culture which I isolated from street dirt gradually lost its putrefying properties when grown on the ordinary laboratory media, so that in a year's time it was unable to make any marked impression on the egg-meat mixture or plain coagulated egg albumin. Eventually it refused to grow in any of the common media, and I was unable to resuscitate it. Dr. Herter informed me that he had had somewhat similar experience with the putrificus bacillus. On the other hand, all the strains of the bacillus of symptomatic anthrax and the bacillus of malignant edema seemed to retain their putrefactive property, and the organisms appeared to be more hardy and long-lived.

In an extended investigation as to the comparative properties of *B. edematis maligni* and *B. anthracis symptomatici*, I have been unable to find any constant differential characters aside from their respective pathogenicity. This is particularly true regarding the nature and rate of putrefaction and fermentation, and the products formed. The morphology and biochemical characters of these organisms as well as of the putrificus bacillus are quite variable, as Achalme¹ and others have shown, and in order to obtain analogous results they must be grown in the same media and under the same conditions. In the egg-meat mixture which I have been employing regularly, the morphology and biochemical properties are quite constant. Here the putrificus organism always assumes the long slender bacillus or drumstick form, the spores being quite large, almost perfectly round and

¹ Achalme: *Ann. de l'Inst. Pasteur*, xvi, p. 633, 1902.

situated at the extremity of the bacillus. In the same medium the rods of the malignant edema and the symptomatic anthrax bacillus are much shorter and thicker, and are located not at, but towards, the end of the bacilli, giving the latter an almost spindle-shaped appearance. When free the spores are seen to be much smaller and decidedly less round than those of *B. putrificus*.

An agar culture of an anaërobe which was sent to the laboratory, labelled *B. botulinus*, Kral, appeared in all of the above-mentioned characters to be identical with the malignant edema and the symptomatic anthrax bacillus. Its putrefactive and fermentative properties were as marked as those of the two other organisms; and as to its morphology, it would certainly have been difficult to distinguish it from them. Whether this organism was the one which was originally isolated as *B. botulinus*, and not a contamination form, it is impossible to state.

The question as to whether the *Bacillus aërogenes capsulatus* of Welch has any pronounced putrefactive action on proteids is an important one. I have examined fully ten strains of this obligate anaërobe which were either obtained from some of the prominent laboratories of this country or directly isolated from feces. Their action was tested on egg-meat mixture, coagulated egg and serum albumin and casein. While there appeared to be a slight decrease in the bulk of the proteids in some instances, as well as the production of quite disagreeable odors, the transformation never assumed the character of real putrefaction. The unpleasant odor which was observed was not like that of mercaptan, etc., but was probably due to the presence of butyric and closely-allied acids. Such decomposition of the medium may take place in the absence of carbohydrates, the proteids themselves undergoing some change, as has been shown by other investigators.

Tissier and Martelly¹ isolated an anërobe from putrefying meat which resembled the Welch bacillus, *B. perfringens*, and which they claimed had a decided putrefying action on blood fibrin. From their paper we are led to believe, however, that the action is not a very rapid one. Herter² also ascribes marked putre-

¹ Tissier and Martelly: *Ann. de l'Inst. Pasteur*, xvi, p. 865, 1902.

² Herter: *Bacterial Infections of the Intestinal Tract*, 1907.

factive properties to the bacillus of Welch and regards it as being largely responsible for certain intestinal disturbances. He uses the term putrefaction in a much broader sense, however, than I do.

Certain investigators (Nencki, Zoya,¹ Kerry, Bienstock) have observed that indol and skatol are absent from the products of the putrefying anaërobes. Passini makes an exception to this rule and asserts that the bacillus of gaseous phlegmon produces an abundance of indol when grown in pure culture on blood serum. I have been unable to find any indol whatever by the usual nitric acid test among the products of *B. putrificus*, *B. œdematis maligni* and *B. anthracis symptomatici*. By the use of Herter's β -naphthaquinone-sodium-monosulphonate test, however, minute traces of indol were detected on different occasions. It appears quite certain that the production of indol is not an important function of the putrefying organisms or anaërobes in general.

PUTREFACTION IN THE HUMAN INTESTINE.

Since Bienstock's assertion¹ that *B. putrificus* is never present in the feces of normal individuals, the subject of intestinal putrefaction has been investigated with renewed effort.

Salus² states that he was able to find spores of putrefying anaërobes, but only in small numbers. He therefore believes that while the organisms may exist in the intestine, they are there in spore form and do not undergo multiplication until after they leave the body.

Tissier and Martelly³ claim to have found and isolated an anaërobe similar to Bienstock's *putrificus* bacillus from meconium. Passini⁴ also seems to have detected the same organisms in meconium, on certain occasions. In the stools of breast-fed infants *B. putrificus* could be found only when several loopfuls of material were taken for examination. Very few spores were present. In bottle-fed children this anaërobe was slightly more

¹ Bienstock: *Arch. f. Hyg.*, xxxix, pp. 390-427, 1901.

² Salus: *Arch. f. Hyg.*, li, p. 97, 1904.

³ Tissier and Martelly: *Ann. de l'Inst. Pasteur*, xvi, p. 865, 1902.

⁴ Passini: *Zeitschr. f. Hyg.*, xlix, p. 135, 1905.

common than in the breast-fed, and in the normal adult the organism occurred in abundance, particularly in the form of spores.

According to Mace¹ the bacillus of malignant edema is a constant inhabitant of the intestinal tract. Passini was unable to substantiate this.

In a more recent publication Bienstock² announces that he was again unable to find any spores of *B. putrificus* in the feces of normal persons, as well as a large number of hospital patients. By the use of a special medium, however, he found an organism resembling his putrificus bacillus in many respects, but differing from it in that it readily attacks sugars with the formation of acetic, butyric, lactic and carbonic acids. It has marked putrefactive and fermentative properties, and was found by Bienstock in 20 per cent of the stools examined. He gave it the name of *B. para-putrificus*.

In my previous paper on putrefaction I mentioned the fact that on two or three occasions organisms were seen in egg-meat cultures made from human feces which in their biochemical aspects resembled *B. putrificus* and the bacillus of malignant edema. Since these organisms are so abundant in nature (dust, dirt, soil, etc.) and since they were seen in only a few samples of the feces examined, it was concluded that they were either present incidentally or were mere contamination forms.

Since then I have examined at least fifty specimens of what might be called normal feces. An effort was made to determine the extent to which native proteids were decomposed, and in the second place to detect one or more of the well-known putrefactive anaërobes which were responsible for this decomposition. To this end the following method was employed.

Known quantities of fresh feces were introduced into large test tubes containing 10 to 12 cc. of the egg-meat mixture previously described.³ After distributing the fecal matter as thoroughly as possible by means of a stout platinum wire, the tubes were heated at 80° C. for 10 minutes, rendered anaërobic by the Wright method and incubated at 37° C. The tubes were

¹ Quoted from Passini, *loc. cit.*

² Bienstock: *Ann. de l'Inst. Pasteur*, xx, p. 497, 1906.

³ Rettger: *This Journal*, ii, p. 71, 1906.

daily examined for signs of putrefaction, but they were not opened until the sixth or seventh day. Experience soon showed that in this medium the putrefying anaërobes could usually be detected between the fourth and seventh day, by their characteristic spore forms. At other times it may be difficult to detect them.

The amount of fecal matter used was roughly estimated by means of three "standard" platinum loops which were made and reserved for that purpose. The smallest loop (*a*) held approximately 2 milligrams of feces; the second (*b*) about 8 milligrams, and the largest (*c*) 32 milligrams. In this way there was less chance of contamination than if weighed quantities of material had been taken and suspended in water or salt solution. The results of some 54 examinations are summed up in the following table:

TABLE SHOWING THE REDUCTION OF SOLID MATTER IN PER CENT, AND THE PRESENCE OF *B. PUTRIFICUS* AND ORGANISMS OF THE MALIGNANT EDEMA BACILLUS TYPE.

Feces	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<i>a</i> (2mg.)	20	40	12	30	0	0	0	0	0	0	0	0	8	0	10	0	10	5
<i>b</i> (8 mg.)	15	40	50†	40	35†	15	0	0	0	0	60*	30	7	60*	30	8†	10	10
<i>c</i> (32 mg)	10	60*	15	75†	0	35†	9	6†	40†	0	?	50*	15	25†	35†	10†	15†	10

†Indicates the presence of *B. putrificus*.

*Indicates the presence of *B. edematis maligni*.

A glance at the table will show that the putrificus bacillus was found in 11 samples, or 20 per cent of all the tubes examined; and bacilli of the malignant edema type in 4 specimens, or 7.4 per cent of the tubes examined. It is quite probable that these figures are too low, as the organisms in question may have escaped detection in some of the tubes, particularly where the reduction in the amount of insoluble proteid was so large.

It will be seen, too, that there is a marked difference in the three series, *a*, *b* and *c*, and that the results are influenced by the amounts of feces used in the tests. Not one of the 18 tubes in series *a* was found to contain the putrefying organisms mentioned, while two-thirds of the positive results were obtained in the tubes which had received 32 milligrams of feces.

Quite a number of examinations were also made of unheated specimens of feces. It was soon discovered that the results were

less satisfactory than when the samples were heated at 80° C. for 10 minutes in order to destroy other organisms which are less resistant or in non-spore form. The progress of putrefaction was much slower, when in evidence, and in fact a smaller percentage of positive results was obtained than by the heat method.

Numerous attempts were made to isolate the anaërobes. This was comparatively easy, as far as the organisms of the malignant edema bacillus type were concerned; with the putrificus bacillus, however, a great deal of difficulty was encountered, and in all but two attempts the results were unsatisfactory. It is indeed a difficult matter to obtain pure cultures of *B. putrificus* from any source.

Normal stools, therefore, contain anaërobes of the putrificus and malignant edema types, but the number of these organisms is very small, except perhaps in some exceptional cases. They exist in the intestines as spores, in this form resisting the unfavorable conditions which must obtain in the normal human intestine. In certain kinds of disturbances of the digestive tract it is highly probable that the anaërobes take advantage of the new conditions and develop to such an extent as to cause excessive putrefaction.

It is impossible to explain satisfactorily the suppressive influence that the human intestine exerts on the putrefying anaërobes. Bienstock claimed that it was due to the antagonistic action of *B. coli* and *B. lactis aërogenes* to the anaërobes. Tissier and Passini discredit this view, and along with other investigators hold that it is due to the acids that are produced from the sugars present, and to the natural protective action of the walls of the small intestine. That there is a certain antagonism of the colon bacillus to the anaërobes in question, my experiments have clearly shown, but I am forced to admit that this antagonism is not strong enough to explain the occurrence of so few of the putrefactive anaërobes in the normal human intestine.

I am fully convinced that the putrificus bacillus which was found in 20 per cent of the stools examined by me was the original *B. putrificus*, and not the paraputrificus bacillus which Bienstock described in his later publication. Tavel,¹ however, observed an anaërobe in certain cases of appendicitis which

¹ Tavel: *Centralbl. f. Bakt., etc.*, i, xxiii, p. 538, 1898.

seems to answer Bienstock's description of *paraputrificus*. This is Tavel's so-called *pseudotetanus bacillus*.

One other obligate anaërobe appeared to me to be of considerable interest, in connection with the above studies, namely, *B. aërogenes capsulatus* of Welch, or *B. enteritidis sporogenes* of Klein. Tests for this organism were made by the method generally used for its detection. The same quantities of fecal matter as mentioned before (*a*, *b* and *c*) were introduced into tubes containing 8 to 10 cc. of milk. After heating the tubes at 80° C. for 10 to 12 minutes, they were placed under anaërobic conditions and kept at incubator temperature for one to three days. Coagulation of the casein with vigorous gas production as shown by the whipped condition of the cream and coagulum were taken as direct evidence of the presence of the Welch bacillus. In ten cases where the reactions were positive, the rabbit test was made and in every instance but one (which probably failed on account of faulty manipulation) the striking phenomenon of gaseous distension of the animal was obtained. The results of the fermentation tests were as follows:

TABLE SHOWING THE PRESENCE OF SPORES OF *B. AEROGENES CAPSULATUS* IN NORMAL FECES.

Feces	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>a</i> (2mg.)	?	—	—	+	—	—	—	+	—	+	+	—	+	+	+	—
<i>b</i> (8 mg.)	+	+	+	+	—	+	—	+	—	+	+	+	+	+	—	—
<i>c</i> (32 mg.)	+	+	+	+	+	+	—	+	+	+	+	+	+	+	—	+

These results refer only to the spores of the organism. There seems to be considerable evidence that the bacillus is present as such in rather large numbers and that the spores are few as compared with the bacilli. Stained preparations of normal feces show but very few spores, as a rule.

In any work on intestinal fermentation and putrefaction the investigator is confronted with several difficulties. Perhaps the most serious of these is the lack of good methods of identifying, isolating and enumerating the anaërobes in question.

The chief points in the present paper may be summed up as follows:

1. Real putrefaction is the work of strict anaërobes only.

2. *Bacillus tetani* has very little or no putrefactive action on native proteids.

3. The bacillus of malignant edema and of symptomatic anthrax have similar morphological and biochemical properties, and aside from their specific pathogenic action in the lower animals, offer a difficult problem of differentiation.

4. *B. aërogenes capsulatus* is primarily a fermentative organism. It has the ability often to attack native proteids to a slight degree, but the transformation is not one of genuine putrefaction.

5. *B. putrificus* and *B. maligni œdematis* are present in normal feces, but only in a limited degree and in all probability in spore form only. As spores they are able to resist the unfavorable conditions of the human intestine. A knowledge of the relative numbers of putrefactive anaerobes in the intestine of normal individuals and those suffering from certain kinds of intestinal disturbances is of the greatest importance.

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On the thermolability of complement¹⁾.

[From the Pathological Laboratory of Indiana University.]

By **Wilfred H. Manwaring**, Sc. B., M. D.,
Associate Professor of Pathology, Indiana University.

With 1 diagram.

In work with cytotoxic sera, it is often necessary to eliminate the action of the complement, or thermolabile substance. To do this, it is customary to heat the serum to from 55° C to 60° C, for from 30 to 60 minutes.

During the course of experiments on the thermogenesis of auxihemolysins, it was necessary to determine, with some degree of accuracy, the time at which the complement is destroyed, when serum is so heated. In one such experiment, a flask containing about 350 c. c. of normal serum was immersed in a thermostatic water-bath at 59° C, and samples of the serum were removed, at two-minute intervals, and tested for the presence or absence of complement. With much surprise it was noted that the complement was apparently completely destroyed at the end of eleven minutes, in spite of the fact that by that time the contents of the flask had reached a temperature of but about 53° C.

This gave a very different conception of the lability of complement from the conception gained from the routine methods of complement destruction. Experiments were, therefore, undertaken to determine, somewhat accurately, the thermal-destruction point of this substance, at different temperatures.

To do this, 1 c. c. of normal serum was placed in each of a dozen or more small test-tubes, and the test-tubes supported, about an inch apart, in a thermostatic water-bath. At stated intervals, tubes were removed and cooled in ice water. The serum in each tube was then tested for complement.

1) Presented before the Chicago Pathological Society, April 9, 1906. Work aided by the Rockefeller Institute for Medical Research.

In making the test, there was added to each tube an amount of amboceptor (heated hemolytic serum) large enough to give 100 per cent. hemolysis with about a fifth of the complement originally present in the tube. No diminution in hemolytic power would therefore be discernible, till over four-fifths of the complement had been destroyed. After this a further decrease in complement would be made evident by lessened hemolysis.

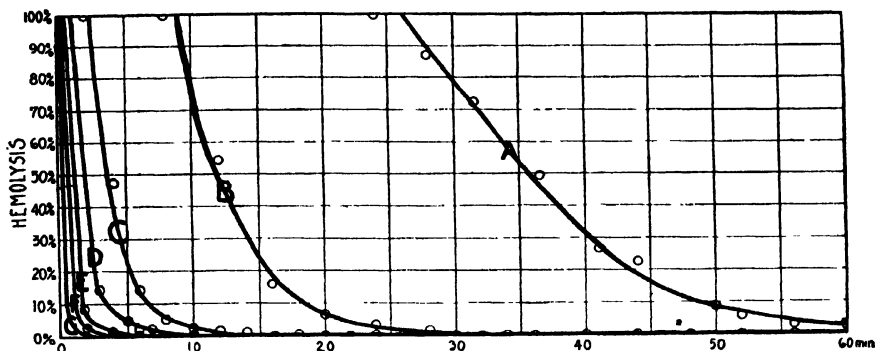


Fig. 1. — The Destruction of Complement by Heat. Curves show loss of the reactivating power of normal goat serum when heated at different temperatures for different periods of time. The experiments so planned that no apparent diminution of hemolytic power would take place, till over 80 per cent. of the complement had been destroyed. A = curve when serum is heated to 49° C; B = curve at 51° C; C at 53° C; D at 55° C; E at 57° C; F at 59° C; and G at 61° C. Curves made with same corpuscles, same sera, and on the same day.

The results of such an experiment are shown graphically in Fig. 1. From this it is seen that, when the serum is heated to 49° C, diminution in hemolytic power becomes evident in about thirty minutes, but that traces of complement remain undestroyed at the end of an hour. When heated to 51° C (B), diminution is evident in ten minutes and apparent complete destruction takes place in thirty-five minutes. At 53° C (C), complete destruction is shown in fourteen minutes; at 55° C (D), in twelve minutes; at 57° C (E), in eight minutes; at 59° C (F), in four minutes; and at 61° C (G), in two minutes.

It is thought that a more careful study of this phenomenon may throw light on the molecular composition of complement.

Summary.

Normal goat serum, heated to 61° C, for two minutes, completely loses its power to reactivate a hemolytic goat serum made inactive by heat. Similar destruction of complement takes place at 59° C, in four minutes; at 57° C, in eight minutes; at 55° C, in twelve minutes; at 53° C, in fourteen minutes; and at 51° C, in thirty-five minutes. At 49° C, complete destruction has not yet taken place in sixty minutes.

107 (250)

The influence of diuresis upon the toxic dose of magnesium salts.

By S. J. MELTZER.

[From the Rockefeller Institute for Medical Research.]

* In the communication on the effects of subcutaneous injections of magnesium salts, John Auer and I stated that a dose of magnesium sulphate slightly larger than 1.75 gram per kilo is surely fatal for the rabbit. Lucas and I showed later that in nephrectomized animals the toxicity of the salts is greatly increased. At the April meeting I conducted an experiment demonstrating that in nephrectomized animals magnesium sulphate can become toxic even when given by mouth. These lines of experimentation have shown that the toxicity of magnesium salts depends upon the normal activity of the kidneys. I wish now to report the results of a series of experiments in which the effect of an increased renal activity was studied.

Briefly stated the results were as follows: A dose of 2 grams of magnesium sulphate per kilo is absolutely fatal for the rabbit; the animal dies of respiratory paralysis in less than an hour. All the animals recovered from the effects of such a dose, however, if an intramuscular injection of diuretin was given soon after the subcutaneous injection of the magnesium salt. Diuretin is theobromin and acts as a diuretic. The deeply narcotized animals usually urinate about fifteen or twenty minutes after its injection; by that time, at least, the bladder can be felt to be full. The largest dose that should be given is about 0.1 gram. In larger doses diuretin itself is liable to become toxic.

In cases in which the dose of the magnesium salts exceeded

2 grams per kilo the injection of diuretin alone could not save the animals. But if in addition to the diuretin an intravenous infusion of 0.9 per cent. solution of sodium chloride was instituted, animals were seen to recover even from doses of magnesium salts amounting to as much as 2.25 grams per kilo. When still larger doses of magnesium salts were given the animals usually died of respiratory paralysis in less than fifteen minutes and before any diuresis could have been effected. However, I have seen animals recover even from doses of 2.5 grams per kilo if, in addition to the diuretin injection and the venous transfusion, artificial respiration was early resorted to. For doses larger than 2.5 grams per kilo all three measures together usually proved of no avail; with this dose the early death of the animal is usually due greatly to paralysis of the heart.

108 (251)

The toxicity of magnesium nitrate when given by mouth.

By **S. J. MELTZER.**

[From the Rockefeller Institute for Medical Research.]

It is a daily experience that large doses of magnesium sulphate can be taken by mouth without any other than a purgative effect. I have given to rabbits, by mouth, 7 grams or more of magnesium sulphate (in molecular solution) per kilo, without any unfavorable effects. The same applies also to magnesium chloride and some other magnesium salts. I have, however, discovered that magnesium nitrate when given by mouth is capable of producing a toxic effect like that of magnesium salts when introduced subcutaneously.

When a dose of 6 grams per kilo in molecular solution is given by mouth to a rabbit, the animal soon becomes paralyzed and narcotized and dies in thirty or forty minutes of respiratory paralysis. Fifteen or twenty minutes after the administration, the appearance and behavior of the animal is exactly like that of one which received magnesium sulphate subcutaneously (2 grams per kilo). A dose between 4 and 5 grams per kilo causes in general the same symptoms but in a gradual way; the animal dies after five or six hours. A dose of between 3 and 4 grams causes no serious effects, but for six or eight hours after its administration the animal

remains in a soporous state ; it sits in one place with eyes closed and head drooping ; a loud noise wakes it up and it attempts to move about or to eat, but in a few minutes it falls asleep again.

This toxicity of the magnesium nitrate is apparently due to its greater absorption from the gastro-intestinal canal. It is certainly not due to its diminished elimination through the kidneys ; on the contrary it acts in some degree as a diuretic, and, when given by subcutaneous injection, the animal withstands a somewhat greater proportionate dose of the nitrate than of the sulphate or chloride, probably because the nitrate increases somewhat the diuresis. As to the share which the anion, the nitrate end of the compound, may have in the toxic effect, I do not wish to make a positive statement ; but I doubt whether it is of any importance. I studied the toxic effects of sodium nitrate administered by mouth and compared the manifestations with those seen after administration of magnesium nitrate ; the contrast was sharp. Even with a dose of 12 grams of the sodium nitrate per kilo there is never such an anesthesia or paralysis as that caused by the magnesium salts ; on the contrary the animal is all excitement and restlessness. Besides, the late death of the animal after administration of sodium nitrate is due to circulatory disturbances, whereas after poisoning with magnesium salts, the animal dies of respiratory paralysis.

THE INFLUENCE OF ALCOHOL ON THE METABOLISM OF HEPATIC GLYCOGEN.¹

By WILLIAM SALANT.²

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(Received for publication, August 20, 1907.)

INTRODUCTORY.

Inquiries concerning the accumulation and transformation of glycogen in the liver have, within recent years, led investigators to study the influences exerted on these hepatic processes by various poisons when introduced in the body. Metallic compounds, also substances of the aliphatic and aromatic series as well as bacterial poisons, have been used. The results of such researches indicate that inorganic as well as certain organic poisons cause the removal of glycogen from the liver.

Thus Kaufholz³ has shown that phosphorus poisoning in rabbits causes rapid transformation of glycogen in the liver. Koch⁴ obtained similar results with corrosive sublimate. Kissel,⁵ working in the same laboratory, corroborated the findings of Koch, and also made the interesting observation that the transformation of glycogen induced in the liver by the administration of corrosive sublimate may be inhibited by means of alcohol. Garnier and Lambert⁶ stated that after the intravenous injection of sodium chloride the liver was freed from glycogen. Kriukoff⁷

¹ The results of some of the experiments have already been communicated in preliminary reports: *Proceedings of the Society for Experimental Biology and Medicine*, 1905-06, iii, p. 58; *Journal of the Am. Med. Assn.*, xlvii, p. 1467, 1906.

² Research Fellow of the Rockefeller Institute.

³ Kaufholz: Dissertation, Würzburg, 1894.

⁴ Koch: *ibid.*

⁵ Kissel: *Centralbl. f. inn. Med.*, xvi, p. 613, 1895.

⁶ Garnier and Lambert: *Compt. rend. de la soc. de biol.*, xlix, p. 617, 1897.

⁷ Kriukoff: Dissertation, Moskau, 1902.

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carried out a large number of experiments with various substances to test their action in this regard. He reported that after subcutaneous injection of arsenic, phosphorus, corrosive sublimate, anilin, phenyl hydrazine, pyrogallol, sulphuric acid,¹ sodium hydroxide, or carbolic acid, glycogen disappeared from the liver in twenty-four hours. Alcohol had the same effect if injected subcutaneously at intervals for a long period.

In this connection the work of Drummond and Noel Paton¹ may be mentioned. These investigators found that in acute adrenalin poisoning in rabbits the liver glycogen was markedly diminished. The same results were obtained by Doyen and Kareff² with adrenalin chloride when they injected this substance into the portal vein. Likewise pilocarpine may, according to these investigators, favor glycogen transformation in the liver. Mohr's³ studies with various gastrointestinal irritants, such as aloin, arsenious acid and croton oil have led to the same conclusion.

Claude Bernard⁴ was the first to announce that during fever the glycogen in the livers of animals decreased even if nourishment was given. His observations were confirmed by Bouley.⁵ May⁶ carried out related experiments on dogs and rabbits. He also found that in fever hepatic glycogen diminishes. May stated that fifteen hours after feeding cane sugar to rabbits in which fever was induced by injecting pathogenic bacteria, 1.69 to 5.05 per cent of glycogen was obtained from the livers of such animals. In control rabbits, which received the same amount of cane sugar, the quantity of glycogen in the livers varied between 9.18 and 11.93 per cent. His results were even more marked when twenty-four hours were allowed to elapse after feeding 30 grams of glucose to rabbits in febrile condition. The liver removed at the end of this time contained an average amount of 0.42 per cent of glycogen. The controls contained 2.71 per

¹ Drummond and Paton: *Journ. of Physiol.*, xxi, p. 92, 1904.

² Doyen and Kareff: *Compt. rend. de la soc. de biol.*, lvi, p. 716, 1897.

³ Mohr: Dissertation, Würzburg, 1894.

⁴ Bernard, Claude, quoted by Roger: *Arch. de physiol. norm. et pathol.*, 5th series, vi, p. 64, 1894.

⁵ Bouley: *ibid.*

⁶ May: *Zeitschr. f. Biol.*, xxx, p. 48, 1894.

cent of glycogen. Ott¹ obtained similar results. He likewise induced infection in rabbits, by the method previously employed by May. Cane sugar was then given such rabbits and only those whose temperature was 40° C. were used for the experiments. Fifteen hours later the livers of these rabbits were removed and examined for glycogen. An average of 5.5 grams of glycogen was found in these livers, while almost double this quantity was found in the livers of the control animals.

That bacterial toxins hasten the disappearance of glycogen from the liver is also made probable by the observations of Luschi² which indicate that the glycogen of the livers of animals brought to a maximum of glycogen accumulation diminishes before the expiration of six hours after infection. Colla,³ who studied the effect of infectious diseases on glycogen, found that glycogen disappears from the liver during tetanus, diphtheria, anthrax or pneumonia. He also examined the livers of a number of children who died of diphtheria. Four, ten or twelve hours after death the livers were free from glycogen. The results obtained by Hirsch and Rolly,⁴ however, do not agree with those just mentioned. After inducing strychnine tetanus (which was preceded by seven days' fasting), 3 cc. of a twenty-four hour bouillon culture of *Bacillus coli communis* were injected into rabbits. Their livers as well as their muscles contained appreciable amounts of glycogen. In one rabbit 0.332 gram of glycogen was found in the liver, which weighed 45 grams. The livers of the control rabbits were free from glycogen.

That some substances, although not glycogen formers, may, nevertheless, favor the accumulation of glycogen in the liver has been indicated by experiments with antipyretics and narcotics. Lepine and Porteret⁵ carried out a large number of experiments on guinea pigs with antipyrin, acetanilid, quinine sulphate and sodium salicylate. They found 20 per cent more glycogen in the livers of these animals than in the controls. The investigations of Nebelthau corroborated these results.

¹ Ott: *Deutsch. Arch. f. klin. Med.*, lxxi, p. 267, 1901.

² Luschi: *Jahresbericht für Thierchemie*, xxx, p. 449, 1900.

³ Colla: *Archiv. Ital. de biologie*, xxvi, p. 120, 1896.

⁴ Hirsch and Rolly: *Jahresbericht für Thierchemie*, xxxiii, p. 604, 1903.

⁵ Lepine and Porteret: *Compt. rend. de l'acad. de sci.*, cvi, p. 1023, 1888.

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After subcutaneous injection of kairin, antipyrin or quinine into hens on the fifth or seventh day of fasting, he found 1.81 per cent to 3.66 per cent of glycogen in the livers. His work, with sulphonal, urethane, chloralamid and paraldehyde, injected on the seventh day into fasting hens, likewise indicated the accumulation of glycogen in the livers. That paraldehyde exerts a similar effect in rabbits was shown by Nebelthau¹ and Cremer.² Nebelthau³ also carried out a series of observations on the effects of ether, chloroform and alcohol on the metabolism of hepatic glycogen in hens. The results he obtained with these substances led him to the conclusion that they favor the accumulation of glycogen in the liver, although in his experiments with alcohol he obtained positive results in only four out of eleven experiments.

The discordant results obtained with alcohol by Nebelthau⁴ and Kriukoff⁵ furnished the indication for the present study of the action of that substance on the metabolism of hepatic glycogen. The determination of the action of alcohol in this regard is especially important also because clinicians frequently prescribe its internal administration in infectious diseases. Colla⁶ maintained that resistance to bacterial invasion varies with the amount of glycogen in the liver. Although his results were not corroborated by the observations of Luschi,⁷ the truth of this contention by Colla is made probable by the work of Roger,⁸ who claimed that the glycogen seems to be an important factor in the liver in reducing the toxicity of alkaloids and other poisons of organic nature when these enter the circulation. The conclusions of Roger were disputed by Vamossy.⁹ The evidence, however, which Vamossy brought forward against the view of Roger is not convincing.

¹ Nebelthau: *Zeitschr. f. Biol.*, xxviii, p. 138, 1891.

² Cremer: *Ergebnisse der Physiologie*, i, p. 876, 1902.

³ Nebelthau: *loc. cit.*

⁴ Nebelthau: *loc. cit.*

⁵ Kriukoff: *loc. cit.*

⁶ Colla: *loc. cit.*

⁷ Luschi: *loc. cit.*

⁸ Roger: *Centralbl. f. klinische Medizin*, ix, p. 5, 1888.

⁹ Vamossy: *Archives internationales de pharmaco-dynamie et de therapie*, xiii, p. 208, 1904.

EXPERIMENTAL.

Methods.—The experiments were carried out on full grown healthy rabbits, between the months of December and May. Throughout the experimental period each animal was kept in a metallic cage provided with a wire net bottom and drip pan to allow drainage of the urine. The room was maintained at a practically uniform temperature since, as Luthje,¹ and later Amalgia and Embden² have shown, the metabolism of carbohydrates is influenced by the temperature of the surrounding atmosphere. Before an experiment the animals were kept under observation for several days in the cages. If failure of adaptation to the new condition was manifested by any of these rabbits they were rejected. In this way some assurance was obtained that the subject of each experiment was normal.

When liver was subjected to analysis, the following procedure was always followed: The animal was quickly killed; its liver was rapidly removed and weighed and placed at once in hot 60 per cent potassium hydroxid solution. Glycogen in the livers was isolated by the shorter method of Pflüger.³ The amount of glucose obtained from the glycogen by hydrolysis was determined by Allihn's method. Later in the course of the investigation, for reasons of economy of time, the amounts of copper thrown down by reduction were determined volumetrically by the iodine method. On account of the sharp end point given by the starch iodine reaction, the latter process was preferred to the cyanide method that is recommended by some investigators for the determination of copper.

The introduction of alcohol or glucose into the body was made *per os* through a stomach tube.

Series I. On fasting rabbits, with small preliminary accumulations of hepatic glycogen. The experiments of several workers, which showed the accumulation of glycogen in the liver after the administration of various narcotics and antipyretics, as well as the observations of Nebelthau⁴ on hens which indicated

¹ Luthje: *Beiträge zur chemischen Physiologie und Pathologie*, vii, p. 309, 1906.

² Amalgia and Embden: *ibid.*, p. 310.

³ Pflüger: *Archiv für die ges. Physiol.*, xciii, p. 163, 1902.

⁴ Nebelthau: *loc. cit.*

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similar results in some cases with alcohol, suggested the advisability of carrying out similar experiments with alcohol on other animals. The possible synthesis of glycogen from alcohol was also thought of, for the work of Goddard¹ on dogs has shown that when large doses of alcohol are given, an appreciable amount of aldehyde may be produced in the blood. Again the high calorific value of alcohol and its ready oxidation in the body led to the expectation that even if alcohol failed to induce an accumulation of glycogen in the liver, its hepatic depletion commonly observed during fasting might, under the influence of alcohol, be reduced or perhaps even entirely inhibited. Experiments were therefore carried out on fasting rabbits, which were given 10 cc. of 30 per cent alcohol per kilo daily for four or five days.

EXPERIMENT 1A, FEMALE RABBIT.

Nov. 20,	2	p. m.	Weight	1180	grams.	Received	10	cc.	30	per cent	alcohol	per kilo.
" 21,	4	"	"	1180	"	"	10	"	30	"	"	"
" 22,	2.30	"	"	1120	"	"	10	"	30	"	"	"

Nov. 22, 3.30 p. m. The rabbit was killed. The weight of the liver was 48 grams. Analysis failed to show the presence of glycogen.

EXPERIMENT 2A, WHITE FEMALE RABBIT.

Nov. 20,	2	p. m.	Weight	1280	grams.	Received	10	cc.	30	per cent	alcohol	per kilo.
" 21,	4	"	"	1210	"	"	10	"	30	"	"	"
" 22,	2.30	"	"	1170	"	"	10	"	30	"	"	"
" 23,	10	a. m.	"	1100	"	"	10	"	30	"	"	"

Nov. 23, 10.30 a. m. The rabbit appeared to be exhausted and dying. She was killed soon afterwards. The weight of the liver was 35 grams. In this rabbit also the liver was free from glycogen.

As controls were used two female rabbits (1 and 2, Table I) to which water instead of alcohol was administered by mouth, through a stomach tube on four successive days. At the end of this period they were killed and the content of glycogen in their livers determined.

This analysis, Table I, as well as that for both the alcoholized rabbits, Table II, failed to show the presence of glycogen in the liver of any one of these animals.

Our finding indicates, therefore, that alcohol, in the amounts given, does not favor the accumulation of glycogen in the livers of fasting rabbits; neither was there any manifestation of the sparing effect of fats or carbohydrates commonly ascribed to alcohol.

¹ Goddard: *Lancet*, ii, p. 1132, 1904.

Series II. On fasting rabbits, with normal preliminary accumulations of hepatic glycogen. Since the foregoing general result might be due to the presence of only relatively low proportions of glycogen in the livers of these animals, which were fed on hay, oats and cabbage, previous to the alcohol period, a series of experiments was carried out in which carrots were fed in large quantities during the fore period of three days in order to induce accumulation of normal amounts of glycogen in the livers of the animals selected. Alcohol (approximately 10 cc. of 30 per cent alcohol per kilo) was then administered daily for four, five or six days.

EXPERIMENT 5A, WHITE RABBIT.

Dec. 13	Weight	1300	grams.	Received	15 cc.	30 per cent	alcohol.
" 14	"	1260	"	"	13 "	30 "	"
" 15	"	1210	"	"	13 "	30 "	"
" 16	"	1180	"	"	25 "	30 "	"
" 17	"	1070	"	"	12 "	30 "	"
" 18	"	1100	"	"	12 "	30 "	"

Dec. 18. The rabbit was killed. The weight of the liver was 44 grams. The amount of glucose obtained by hydrolysis of glycogen was 0.92 per cent of the fresh tissue.

EXPERIMENT 6A, WHITE RABBIT.

Dec. 12	Weight	1580	grams.	Received	16 cc.	30 per cent	alcohol.
" 13	"	1540	"	"	15 "	30 "	"
" 14	"	1500	"	"	15 "	30 "	"
" 15	"	1440	"	"	15 "	30 "	"
" 16	"	1400	"	"	25 "	30 "	"
" 17	"	1300	"	"	13 "	30 "	"
" 18	"	1300	"	"	13 "	30 "	"

Dec. 18. The rabbit was killed. The weight of the liver was 48 grams. The amount of glucose obtained by hydrolysis of glycogen was 0.31 per cent of the fresh tissue.

EXPERIMENT 9A, FEMALE RABBIT.

Dec. 24	Weight	1520	grams.	Received	15 cc.	30 per cent	alcohol.
" 25	"	1420	"	"	15 "	30 "	"
" 26	"	1410	"	"	15 "	30 "	"
" 27	"	1350	"	"	13 "	30 "	"
" 28	"	1300	"	"	13 "	30 "	"
" 29	"	1280	"	"	15 "	30 "	"
" 30	"	1220	"	"	19 "	30 "	"

Dec. 30, 2.40 p. m. The rabbit was killed. The weight of the liver was 47 grams. Only a trace of glucose was obtained.

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EXPERIMENT 7A, MALE RABBIT.

Dec. 24 Weight 1670 grams. Received 17 cc. 30 per cent alcohol.
 " 25 " 1700 " " 17 " 30 " "

Several hours after it received the last dose the rabbit escaped from the cage and was found eating hay and oats. The experiment was then continued, as follows:

Dec. 26 Weight 1620 grams. Received 17 cc. 30 per cent alcohol.
 " 27 " 1580 " " 17 " 30 " "
 " 28 " 1480 " " 15 " 30 " "
 " 29 " 1530 " " 20 " 30 " "
 " 30 " 1470 " " 19 " 30 " "
 " 31 " 1500 " " 15 " 30 " "

Dec. 31, 5 p. m. The rabbit was killed. The weight of the liver was 53 grams. The amount of glucose obtained by hydrolysis of the glycogen was 0.09 per cent of the fresh tissue.

EXPERIMENT 9B, GRAY FEMALE RABBIT.

Dec. 24 Weight 1410 grams. Received 15 cc. 30 per cent alcohol.
 " 25 " 1450 " " 15 " 30 " "

(At this point there was a fasting period of the same length and for identical reasons as that for rabbit 7a.)

Dec. 26 Weight 1420 grams. Received 15 cc. 30 per cent alcohol.
 " 27 " 1350 " " 14 " 30 " "
 " 28 " 1370 " " 14 " 30 " "
 " 29 " 1350 " " 15 " 30 " "
 " 30 " 1300 " " 20 " 30 " "
 " 31 " 1270 " " 14 " 30 " "

Dec. 31, 5 p. m. The rabbit was killed. The weight of the liver was 43 grams. Only a trace of glucose was obtained.

EXPERIMENT 10A, GRAY RABBIT.

Jan. 1 Weight 1830 grams. Received 18 cc. 30 per cent alcohol.
 " 2 " 1690 " " 17 " 30 " "
 " 3 " 1540 " " 15 " 30 " "
 " 4 " 1510 " " 15 " 33 " "
 " 5 " 1470 " " 15 " 33 " "

Jan. 5. The rabbit was killed. The weight of the liver was 53 grams. The amount of glucose obtained by hydrolysis of the glycogen was 0.02 per cent of the fresh tissue.

EXPERIMENT 11A, GRAY FEMALE RABBIT.

Jan. 1 Weight 1700 grams. Received 18 cc. 33 per cent alcohol.
 " 2 " 1560 " " 16 " 33 " "
 " 3 " 1460 " " 15 " 33 " "
 " 4 " 1450 " " 15 " 33 " "
 " 5 " 1350 " " 14 " 33 " "

Jan. 5. The rabbit was killed. The weight of the liver was 54 grams. The amount of glucose obtained by hydrolysis of the glycogen was 0.16 per cent of the fresh tissue.

The results obtained in the experiments of this series, which are also shown in Table II, likewise failed to give evidence of any inhibitory action of alcohol on the depletion of hepatic glycogen. Of the three rabbits which received alcohol daily for *six* days, the liver in one contained less than 0.1 per cent of glycogen; in each of the livers of the other two rabbits only traces of glycogen were present at the conclusion of the experiments. In one rabbit, 8, Table I, which was used as a control and was given water by mouth through a stomach tube for the same length of time after preliminary feeding with carrots, the amount of glycogen in the liver was a little more than 0.04 per cent. In this connection it may be pointed out that the livers of two normal rabbits, kept on a diet of carrots for three days, contained 3 to 4 per cent of glycogen at the end of that time.

In two experiments alcohol was given during a period of *five* days. Appreciably larger amounts of glycogen were found in the livers of these rabbits than in the livers of the controls (see Tables 1 and 2, p. 416), which would seem to indicate that alcohol caused retarded transformation of glycogen. This was improbable, however, in the light of the results of Experiments 10a and 11a. In the latter experiments, very small amounts of glycogen were found in the livers of these rabbits, which received alcohol on four days after the usual preliminary feeding of carrots. It was thought, however, that possibly larger quantities of alcohol and consequently an increase in the number of calories might spare the hepatic glycogen. Two experiments were carried out to test this suggestion (Series III).

Series III. Same as Series II, with larger doses of alcohol.

EXPERIMENT 17A, WHITE RABBIT.

Fasted for six days, was then fed carrots on four days (Jan. 30 to Feb. 3.)

Feb.	3	Weight 1800 grams.	Received 20 cc. 30 per cent alcohol.
"	4 3	p.m.	" 20 " 60 " "
"	5 11.30	a.m.	" 10 " 60 " "
"	5 3	"	" 10 " 60 " "
"	6 10	a.m.	" 10 " 60 " "
"	6 3	p.m.	" 12 " 60 " "
"	7 12	noon	" 13 " 60 " "

At 3.30 p. m. the rabbit was found dead, but was still warm. The weight of the liver was 66 grams. A qualitative test failed to show the presence of glycogen in the liver.

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EXPERIMENT 18A, GRAY RABBIT.

Fasted six days. Carrots were given on four days. (Jan. 30 to Feb. 3).

Feb. 3	Weight 1130 grams.	Received 15 cc. 30 per cent alcohol.
" 4 3 p.m.	" 12 " 60	" "
" 5 11 30 a.m.	" 8 " 60	" "
" 5 2.30 p.m.	" 7 " 60	" "
" 6 10 a.m.	" 8 " 60	" "
" 6 3 p.m.	" 8 " 60	" "
" 7 12 noon	" 10 " 60	" "
" 7 8.30 p.m.	" 10 " 60	" "

Feb. 7, 10.30 p. m. The rabbit was killed. The weight of the liver was 45 grams. As in the preceding experiment the liver was free from glycogen.

The results of analysis in these experiments (Series III) likewise indicate that even somewhat larger quantities of alcohol than those of the previous experiments failed to inhibit the disappearance of glycogen from the livers of fasting rabbits, this organ in each rabbit having been found free from glycogen. The question whether alcohol may accelerate the transformation of glycogen in the liver now presented itself with special force.

Series IV. Does alcohol accelerate the transformation of hepatic glycogen. To answer this question various amounts of glucose were given to rabbits by mouth through a stomach tube. Alcohol was administered either immediately after the glucose was given or, as in some experiments, a few hours were allowed to elapse between the last feeding of glucose and the succeeding dose of alcohol. In some experiments two doses of alcohol were administered at intervals of eighteen to twenty-four hours. In one experiment (28a), only one dose was given while another rabbit (29a) received three doses of alcohol. As controls I used rabbits which were fed varying amounts of glucose. The rabbits were killed at the end of different periods following the administration of glucose.

Experiment 24a, white rabbit. Fasted 6 days. Weight, 1800 grams.

March 19, 10 grams of glucose dissolved in water were given by mouth. Immediately afterward the animal received 10 cc. of 60 per cent alcohol.

March 20, 10 grams of glucose were fed, then 15 cc. of 60 per cent alcohol were given.

About sixteen hours later the rabbit was killed. The weight of the liver was 52 grams. The amount of glucose obtained by hydrolysis of glycogen was 1.9 per cent of the fresh tissue.

Experiment 24b, black rabbit. Weight, 1850 grams. Fasted 6 days. March 19, 10 grams of glucose given by mouth.

March 20, 10 grams glucose given by mouth.

Sixteen hours later the rabbit was killed. The weight of the liver was 51 grams. The amount of glucose obtained by hydrolysis of glycogen was 4 per cent of the fresh tissue.

Experiment 25a, rabbit. Weight, 1550 grams. Fasted 6 days.

May 9, 3.30 p.m. Eight grams of glucose given by mouth.

May 9, 10 p.m. Received 15 cc. of 60 per cent alcohol.

May 10, 11 a.m. Weight, 1500 grams. Received 15 cc. of 60 per cent alcohol.

The rabbit was killed May 11 at 3 p.m. About 15 minutes after alcohol had been given, symptoms of intoxication appeared which lasted several hours. At the time of death, the rabbit looked normal. No glycogen was present in the liver.

Experiment 25. Rabbit fasted 6 days. May 10, 10 a.m. Weight 1850 grams. Nine grams of glucose were given by mouth. May 10, 3 p.m. Water was given by mouth. The rabbit was killed May 11 at 7 p.m. The weight of the liver was 33 grams. The amount of glucose obtained by hydrolysis of glycogen was 1.23 per cent of the fresh tissue.

Experiment 26a. Weight of rabbit, 1450 grams. Fasted 6 days.

May 9, 3.30 p.m. Seven grams of glucose, dissolved in water, were given by mouth.

May 9, 10 p.m. Fifteen cc. of 60 per cent alcohol were given by mouth.

May 10, 11 a.m. Weight, 1300 grams. Received 13 cc. of 60 per cent alcohol.

Shortly afterward, the rabbit manifested symptoms of severe intoxication which continued all day. While still under the influence of alcohol, May 10, 7 p.m., the rabbit was killed. The amount of glucose obtained by hydrolysis of the glycogen was 3.33 per cent of the fresh tissue.

Experiment 27a. Rabbit fasted 6 days.

May 9, 4 p.m. Weight, 1200 grams. Six grams of glucose were given by mouth.

May 9, 10 p.m. Received 15 cc. of 60 per cent alcohol.

May 10, 11 a.m. Weight, 1100 grams. Received 11 cc. of 60 per cent alcohol.

May 10, 7 p.m. The rabbit was killed. Signs of intoxication developed ten minutes after the administration of alcohol. At the time of death the rabbit looked normal. The weight of the liver was 36 grams. The liver of this rabbit was free from glycogen.

Experiment 28. The rabbit fasted 6 days.

May 27, 6 p.m. Weight, 1450 grams. Fifteen grams of glucose dissolved in water were given by mouth.

May 28, 12 noon. Weight, 1450 grams. Fifteen grams of glucose were given as before.

May 30. The rabbit struggled a good deal when he was fastened on the holder.

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May 30, 6 p.m. Killed. The weight of the liver was 30 grams. The amount of glucose obtained by hydrolysis of the glycogen was 0.11 per cent of the fresh tissue.

Experiment 29a. Rabbit fasted 6 days.

May 27, 6 p.m. Weight, 1450 grams. Fifteen grams of glucose were given by mouth.

May 28. Weight, 1450 grams. Fifteen grams of glucose were given as before.

May 28, 6.15 p.m. Received 15 cc. of 60 per cent alcohol.

May 29, 5 p.m. Received 10 cc. of 60 per cent alcohol.

May 30, 11 a.m. Weight, 1450 grams. Received 10 cc. of 60 per cent alcohol.

May 30, 4.30 p.m. The rabbit was killed while apparently still under the influence of alcohol. The weight of the liver was 44 grams. Analysis did not show the presence of glycogen.

Experiment 30a. Rabbit fasted 6 days.

May 27, 6 p.m. Weight, 1270 grams. Received 13 grams of glucose dissolved in water.

May 28, 12.15 p.m. The same amount of glucose was given.

May 29, 5.30 p.m. Received 12 cc. of 60 per cent alcohol.

May 30, 11 a.m. Received 15 cc. of 60 per cent alcohol.

May 30, 4.30 p.m. The rabbit was killed while still under the influence of alcohol. This liver was likewise free from glycogen.

Experiment 31a. Rabbit fasted 6 days.

May 27, 6 p.m. Weight of rabbit, 1800 grams. Eighteen grams of glucose were given. This was repeated next day. Fifty-two hours later the rabbit was killed. The liver was removed and treated as in the other experiments. The weight of the liver was 28 grams. The amount of glucose obtained from the glycogen was 1.24 per cent of the fresh tissue.

Experiment 28a. Rabbit fasted 6 days. Weight, 1230 grams.

May 27 and 28. Thirteen grams of glucose were administered as in previous experiments.

May 28, 6 p.m. Received 13 cc. of 60 per cent alcohol.

May 29, 6 p.m. The rabbit was found in a dying condition. It was then killed. The liver was removed immediately and subjected to analysis by the usual method. Weight, 45 grams. The amount of glucose obtained from the glycogen was 1.24 per cent of the fresh tissue.

The analytic results given in the table (III) show with one exception (No. 26a) a marked diminution in the glycogen of the livers of the alcoholized rabbits as compared with the controls. Thus, sixteen hours after the last feeding of glucose the total amount of glycogen obtained was 3.7 per cent in the control (24), while in the rabbit given alcohol (24a), which received the same amount of glucose (20 grams in twenty-four hours), the quantity

of glycogen found in the liver was 1.76 per cent, less than half that found in the control.

In the next group each rabbit was given a single dose of 5 to 9 grams of glucose per kilo. They were killed twenty-eight hours later. Excepting rabbit 26a this series showed in a striking manner the action of alcohol in hastening the transformation of hepatic glycogen, for, whereas the amount of glycogen in the controls was 1.14 per cent (25) and 2.3 per cent (26), the livers of the two corresponding alcoholized rabbits were free from glycogen. The administration of alcohol after feeding much larger quantities of glucose (20 gm. per kilo in twenty-four hours) was accompanied by similar results when the rabbits were killed in from fifty-two to fifty-four hours after they received the final dose of glucose. In the two controls of this group the amount of glycogen obtained was 0.11 per cent in one (28), and 1.14 per cent (31a) in the other, whereas the livers of the two alcohol rabbits were free from glycogen. In another experiment of this group (28a) the rabbit received the same amount of glucose per kilo in twenty-four hours, but, as he was in a dying condition, apparently from the effects of alcohol, he was killed thirty hours after he had received the final dose of glucose. The liver of this rabbit contained only 1.33 per cent of glycogen, which is only 0.2 per cent more than that found in the control rabbit (25) and about 1 per cent less than in rabbit 26, which received one-fourth the amount of glucose per kilo fed to rabbit 28a.

The discrepancy between the results obtained for rabbits 26a, and in the other alcohol experiments of the same series, may be explained by the following observations: It was noted that in this rabbit symptoms of severe alcohol intoxication (which set in soon after the second and final dose of alcohol was given) persisted eight hours, while the other rabbits of this group similarly treated behaved at the end of this time as if they had recovered completely from the effects of alcohol intoxication. This difference in the reaction to alcohol is suggestive of the possibility that during the stage of profound alcohol narcosis, glycogen metabolism is not affected. Perhaps it is only when recovery from narcosis is practically complete that metabolism of glycogen is resumed.

In the two alcohol rabbits 29a and 30a, each of which was killed in a state of deep intoxication, this supposition apparently

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TABLE I. AMOUNTS OF GLYCOGEN IN THE LIVERS OF CONTROL RABBITS.

Exp. No.	Rabbit.	Liver.	Food before fasting.	Fasting period.	Treatment during fasting.	Hepatic glycogen: per cent of the fresh tissue calculated from glucose.
	gms.	gms.		days		
1	820	22	C. H. O.*	4	Water given by stomach tube.	none
2	1320	41	"	5		"
5	1230	27	Carrots 3 days.	5		0.139
6	970	22	"	5		0.148
8	1370	42	"	6		0.043
10	1265	38	"	4		0.127
11	1470	53	"	4		none

TABLE II. AMOUNTS OF GLYCOGEN IN THE LIVERS OF FASTING RABBITS AFTER ADMINISTRATION OF ALCOHOL.

Exp. No.	Rabbit.	Liver.	Food before fasting.	Fasting period.	Alcohol per kilo daily.	Hepatic glycogen: per cent of the fresh tissue calculated from glucose.
	gms.	gms.		days		
1a	1120	48	C. H. O.*	4	30% in cc.	none
2a	1100	35	"	5		"
5a	1100	44	Carrots 3 days	5		0.84
6a	1300	48	"	5	10	0.28
9a	1280	47	"	6	10	trace
7a	1500	53	"	6	10	0.083
9b	1270	43	"	6	10	trace
10a	1470	53	"	4	10	0.018
11a	1350	54	"	4	10	0.148
3a	800	35	C. H. O.	4	12	trace
17a	1800	66	Carrots 3 days	3½	60% in cc.	none
18a	1130	45	"	4		none

* C. H. O.—Cabbage, hay, oats.

was not borne out by the results of the analysis. As shown in the table, their livers did not contain any glycogen. The protocols show, however, that the intervals between the two successive doses of alcohol in each case were eighteen or twenty-four hours, thus giving sufficient time for recovery from its intoxicating effect. In rabbit 26a, the interval was only twelve hours, which may account for the different results obtained.

TABLE III. SHOWING THE EFFECTS OF COMPARATIVELY LARGE QUANTITIES OF ALCOHOL ON THE METABOLISM OF HEPATIC GLYCOGEN.

Exp. No.	Rabbit.	Liver.	Glucose fed per kilo in 24 hours.	60% alcohol per kilo daily.	No. hours between last feeding of glucose and death.	Hepatic glycogen: per cent of the fresh tissue calculated from glucose.
	gms.	gms.	gms.	cc.		
24	1850	51	20	0	16	3.70
24a	1800	52	20	25	16	1.76
25	1850	33	9	0	28	1.14
25a	1550	48	8	30	28	
26a	1450	58	7	28	28	3.08
26	1120	41	5½	0	28	2.30
27a	1200	36	6	26	28	
28	1450	30	29	0	54	0.11
29a	1450	44	29	35	52	
30a	1270	41	26	27	52	
31a	1800	28	36	0	52	1.14
28a	1230	45	25	13	30	1.33
21a	1050	41	32	20	12	8.65
23a	815	35	25	12	7	9.17

The suggestion that alcohol does not hasten the transformation of glycogen of the liver during the stage of profound narcosis gains further support from the results of experiments 21a and 23a, in which large quantities of glucose were followed by alcohol (shortly after, in one experiment, or three hours later, in another). The amounts of glycogen found six to twelve hours after the final dose of glucose had been given were 9.17 per cent and 8.65 per cent, respectively.

It is to be noted that in both these experiments the rabbits were killed from six to eight hours after receiving large amounts of alcohol in proportion to body weight, that is, before the stage of intoxication passed off. The conclusion seems to be justified, therefore, that large quantities of alcohol may hasten the process by which glycogen is made to disappear from the liver and that it apparently exerts this action only after the stage of intoxication has been passed. This mode of action of alcohol might explain the discordant results of Nebelthau's experiments, some of which showed disappearance of hepatic glycogen, while others indicated the presence of considerable amounts, after administration of alcohol. It may in the same way also account

for the different results obtained by Nebelthau and Kriukoff, whose work was referred to on p. 406.

Objection to this conclusion may be raised on the ground that there is a wide range of variation in the proportion of glycogen found in the livers of the control rabbits. Thus in experiment 28a, only 0.11 per cent was obtained, while in 31a, the presence of 1.14 per cent of glycogen was shown. This difference may possibly have been due to the activity of rabbit 28a, when it was tied on the holder before it was killed. The other rabbits did not behave in this way. Again, in experiments 25 and 26 (two control rabbits) there was a difference of 50 per cent in the liver glycogen found; as great a difference as there was between the alcohol rabbit in experiment 24a, and the corresponding control. The wide variation in the amount of hepatic glycogen of these two normal rabbits does not invalidate the above conclusions, since in the alcoholized rabbits of the same group (25a and 27a), which received as much glucose per kilo as the controls, and were killed at the same time after feeding glucose, the livers were free from glycogen. Moreover, the difference between the amounts obtained from the two normal rabbits may possibly be accounted for by a difference in muscular activity. It is of importance to note, in this connection that, in the alcoholized rabbits, intoxication and consequent loss of muscular power set in about 15 minutes or sometimes even earlier after alcohol was administered, which would tend to inhibit rather than hasten the amyolysis.

That other toxic substances may exert an action on hepatic glycogen comparable to that here attributed to alcohol was shown by Roger.¹ He was the first to find that anthrax has no effect on glycogen during the first stage of infection, but later accelerates its disappearance from the liver.

I am very much indebted to Prof. William J. Gies for valuable suggestions received in the course of this investigation.

¹ Roger: *Arch. de physiol. norm. et pathol.*, 5th series, vi, p. 64, 1894.

Über die Analyse der Spaltungsprodukte des Eialbumins.

Von

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Es wurde schon in einer früheren Mitteilung erwähnt, daß es für das Studium des Verdauungsprozesses des Proteins wichtig ist, ein Verfahren auszuarbeiten, welches die Analyse ohne Anwendung der Fischerschen Estermethode ermöglichte. In der erwähnten Mitteilung wurde es über die Analyse der Spaltungsprodukte der Gelatine berichtet¹⁾, nur unterscheidet sich dieser Körper in seiner Zusammensetzung von anderen Proteinen so bedeutend, daß man es nicht erwarten konnte, daß ein Verfahren, welches die Ausführung der Analyse in diesem Falle ermöglichte, auch für andere Proteine brauchbar sein würde. Es wurde deshalb versucht, die Analyse der Spaltungsprodukte des Eialbumins durchzuführen. Ganz unerwartet stieß man auf große Schwierigkeiten bei dem Versuche, reines Leucin und reines Tyrosin darzustellen. Diese Schwierigkeiten wurden aber nach einigen Bemühungen überwunden. Das Verfahren beruhte auf den folgenden Eigenschaften der einzelnen Aminosäuren.

Glutaminsäure wurde isoliert nach der Methode von Halseewitz und Habermann, welche jüngst wieder von Osborne und Gilbert¹⁾ in Gebrauch genommen. Analysenreines Tyrosin wurde durch Fällung mittels Bleizuckerlösung hergestellt. Aus der Mischung von Leucin und Aminovaleriansäure wurde die erste durch Fällung mittels Bleizucker und Ammoniak ermöglicht

¹⁾ Amer. Journ. of Physiol. 15, 333, 1906.

und die letztere durch Kristallisieren aus 75% Essigsäure. Das Phenylalanin wurde als Phosphorwolframat getrennt, das Prolin erhielt man als alkohollösliches Kupfersalz. Oxyprolin gelang es nicht zu gewinnen nach dem Verfahren, wie es bei der Untersuchung der Gelatine ausgeführt wurde, — man erhielt dabei eine Substanz, die scheinbar nicht ganz reines Serin war. Glykokoll und Alanin wurden als Phosphorwolframate gefällt und voneinander durch alkoholische Pikrinsäurelösung getrennt. Die Asparaginsäurefraktion wurde durch Unfall verloren, nachdem die Anwesenheit der Substanz in der betreffenden Fraktion erwiesen worden war. Die Einzelheiten des Verfahrens folgen:

400 g trockenes Eialbumin kamen zur Analyse. Sie wurden mit 1500 ccm konzentrierter Salzsäure 5 Stunden lang mit Rückflußkühler erhitzt, die Lösung dann bis etwa auf 200 ccm eingedampft und in einem Refrigerator bei -1°C mehrere Tage lang stehen gelassen. Die salzsaure Glutaminsäure wurde durch Saugepumpen abfiltriert, dann in Wasser aufgelöst, mit Bariumcarbonat alkalisch gemacht und erhitzt, um den vorhandenen Ammoniak zu verjagen. Das Barium wurde dann quantitativ entfernt, die Lösung eingedampft, mit Tierkohle entfärbt und zum Kristallisieren stehen gelassen. Ein Teil des Hydrochlorates der Glutaminsäure wurde in die freie Säure übergeführt und analysiert. Die Ausbeute betrug 45,0 g.

Die Analyse des im Xylolbad getrockneten Präparates ergab die folgenden Zahlen:

0,1352 g der Substanz gaben 0,1985 g CO_2 und 0,0147 g H_2O für $\text{C}_5\text{H}_9\text{N}_2\text{O}_4$

Berechnet	Gefunden
C = 40,81%	40,95%
H = 6,12%	6,14%

Das Filtrat von Glutaminsäure wurde mit Wasser bis auf 8 l verdünnt und mit einer 10prozentigen Lösung von Phosphorwolframsäure so lange behandelt, bis noch ein Niederschlag, der aus den Hexonbasen bestand, sich bildete. Das Filtrat wurde auf übliche Weise von Phosphorwolframsäure und von Salzsäure befreit und zur Ausscheidung des Tyrosins, Leucins, Phenylamins und der Aminovaleriansäure eingedampft. Es ist kaum

möglich, durch irgend welche Methode diese Säuren quantitativ voneinander zu trennen. Die Angaben über die Ausbeuten an einzelnen Substanzen müssen deshalb nur als annähernd richtige betrachtet werden. Die Ausbeute der vier Säuren betrug 80 g.

Diese Menge wurde wieder durch ihre Löslichkeit in Wasser in drei Fraktionen getrennt. Die erste, die zur Darstellung des reinen Tyrosins bestimmt wurde, betrug 6 g. Eine mittlere Fraktion, zur Darstellung des Leucins und Aminovaleriansäure, betrug 35 g, und die dritte, aus welcher Phenylalanin isoliert wurde, enthielt 40 g.

Zur Gewinnung des reinen Tyrosins wurde die Fraktion in heißem Wasser aufgelöst und heiß mit Bleizucker gefällt und bald filtriert. Der Niederschlag durch Schwefelwasserstoff zersetzt, das Tyrosin kristallisieren gelassen. Man erhält ein Präparat, welches ohne Umkristallisieren die theoretische Zusammensetzung des Tyrosins besitzt.

0,1648 g der Substanz gaben 0,3588 g CO_2 und 0,0943 g H_2O für $\text{C}_9\text{H}_{12}\text{NO}_3$.

Berechnet	Gefunden
C = 59,62%	59,51%
H = 6,13%	6,35%

Die mittlere Fraktion wurde in möglichst wenig heißem Wasser gelöst, mit 20% Bleizuckerlösung behandelt, wobei eine opalisierende Lösung entsteht, zu dieser wird Ammoniak so lange zugegeben, bis noch ein Niederschlag entsteht, die Mischung zum Kochen erhitzt und filtriert. Der Niederschlag, von Blei befreit, bildet analysenreines Leucin.

0,1732 g des im Toluolbad getrockneten Präparates gaben 0,3501 g CO_2 und 0,1556 g H_2O für $\text{C}_6\text{H}_{13}\text{NO}_2$.

Berechnet	Gefunden
C = 54,89%	55,12%
H = 10,01%	9,9%

Die Substanz enthielt noch scheinbar auch Isoleucin.

Das Filtrat von Bleileucin wurde vom Blei befreit, die Lösung zum Kristallisieren überlassen; die wenig lösliche Fraktion entfernt und der Rest zur Trockne eingedampft. Diese Substanz

wurde dann aus 80% Essigsäure umkristallisiert. Es entstand dabei eine Substanz, die die Zusammensetzung der Amino-valeriansäure besaß.

0,1500 g der Substanz, im Toluolbad getrocknet, gaben 0,2828 g CO_2 und 0,1266 g H_2O .

Für $\text{C}_8\text{H}_{11}\text{NO}_2$

Berechnet	Gefunden
C = 51,22%	51,6%
H = 9,48%	9,38%

1 g der Substanz wurde in 20 ccm Wasser aufgelöst und mit 5 g umkristallisiertem Bariumhydrat für 20 Stunden im Autoklav bei 175° erhitzt.

Die Lösung wurde von Barium befreit und nach Neuberg und Manasse¹⁾ mit α -Naphthylisocyanat behandelt. Es entstand dabei ein Produkt, welches nach mehrmaligem Umkristallisieren aus verdünntem Alkohol bei 169 — 170° schmolz. (Leucin gibt nach Neuberg und Manasse ein Hydantoin mit S. P. = 167 und Aminovaleriansäure nach Osborne und Clapp eines mit S. P. = 181° C. Dagegen ist es uns gelungen, eine α -Naphthylhydantoinssäure mit S. P. = 179 — 180° aus inaktivem Leucin zu erhalten.)

Die dritte Fraktion wurde in möglichst wenig 5% Schwefelsäure aufgelöst und mit einer konzentrierten Lösung von Phosphorwolframsäure (4 Teile Säure auf 1 Teil Wasser) präzipitiert. Es bildete sich zuerst ein ölartiger Niederschlag; das Reagens wurde so lange zugeführt, bis sich das Öl wieder auflöste; die Lösung wurde dann im Eisschrank stehen gelassen; es bildete sich dann ein Niederschlag, der aus rohem Phenylamin bestand. Die Ausbeute betrug 8 g. Die Substanz hatte 62,6% C und 6,4% H.

Zur Reinigung wurde sie wieder in 5% Schwefelsäure aufgelöst und wieder mit Phosphorwolframsäure behandelt, dabei entstand das typische Phosphorwolframat des Phenylamin, wie es von Schulze und Winterstein beschrieben war.

Dieses Phosphorwolframat wurde wieder in die freie Substanz auf übliche Weise umgewandelt und analysiert.

¹⁾ Ber. d. deutsch. chem. Ges. 38, 2359, 1905.

0,1075 g der Substanz gaben 0,2578 g CO_2 und 0,0639 g H_2O .
Für $\text{C}_9\text{H}_{12}\text{NO}_2$

Berechnet	Gefunden
C = 65,40%	65,32%
H = 6,73%	6,60%

Das Filtrat der Tyrosin-Leucinfraction wurde für die Analyse der anderen Aminosäuren verbraucht. Zu diesem Zwecke wurde die Substanz in viel Wasser aufgelöst und mit einem möglichst kleinen Überschuß von Kupferoxyd gekocht.

Das Filtrat von diesem bei vermindertem Druck eingedampft und dann in drei Fractionen getrennt.

Erste löslich in absolutem Alkohol, zweite löslich in 75% Alkohol und dritte unlöslich in letztem Reagens.

Die erste Fraction wurde auf Prolin verarbeitet, die zweite auf Oxysäuren und die dritte auf Glykokoll, Alanin und Asparaginsäure.

Zum Erhalten des Prolins entfernte man aus der ersten Fraction das Kupfer, konzentrierte dann die erhaltene Lösung auf ein möglichst kleines Volumen, bestimmte den Gehalt an Stickstoff und racemisiert durch Erhitzen im Autoklav mit der berechneten Menge von Bariumhydrat. Die Lösung wurde dann zur Trockne eingedampft und wieder mit absolutem Alkohol extrahiert. Dieser Auszug wurde dann verbraucht zur Darstellung des Kupfersalzes des inaktiven Prolins. Die Ausbeute betrug 1,60 g.

0,7876 g der Substanz gaben 0,0086 g H_2O .
Für $(\text{C}_5\text{H}_9\text{NO}_2)_2\text{CuH}_2\text{O}$

Berechnet	Gefunden
$\text{H}_2\text{O} = 10,0\%$	10,93%

Aus der Fraction der Kupfersalze, löslich in 75% Alkohol, wurde bei der Zersetzung der Gelatine das Oxyprolin erhalten. Auf ähnliche Weise behandelt, gab aber diese Fraction bei Eialbumin kein Oxyprolin, sondern eine Substanz, die scheinbar aus unreinem Serin bestand.

0,1777 g der Substanz gaben 0,2418 g CO_2 und 0,1066 g H_2O .
Für $\text{C}_3\text{H}_2\text{NO}_3$

Berechnet	Gefunden
C = 34,28%	36,5%
H = 6,66%	6,66%

Die dritte Fraktion der Kupfersalze wurde von Kupfer befreit, in möglichst wenig 5prozentiger Schwefelsäure gelöst und mit großem Überschuß von Phosphorwolframsäure (4 Teile Säure in 1 Teil Wasser) behandelt. Die Lösung wurde im Eisschranke bei -1°C stehen gelassen und von dem sich gebildeten Niederschlage befreit. Dieser wurde von der Phosphorwolframsäure befreit und bestand nun aus 10,0 g Glykokoll und Alanin. Die Substanz wurde in möglichst wenig Wasser gelöst und mit alkoholischer Pikrinsäure behandelt und über Nacht im Eisschranke bei -1°C stehen gelassen. Das Glykokollpikrat wurde aus möglichst wenig Wasser umkristallisiert und analysiert.

0,1200 g der Substanz gaben 19,5 ccm Stickstoff (über 50% KOH) bei $= 23,5^{\circ}\text{C}$ und $p = 760$.

Für $\text{C}_2\text{H}_5\text{NO}_2\text{C}_0\text{H}_2(\text{NO}_2)_3\text{OH}$

Berechnet	Gefunden
N = 18,35%	18,20%

Das Filtrat von Glykokollpikrat wurde von Pikrinsäure mittels Schwefelsäure und Äther befreit, die Schwefelsäure wurde dann entfernt und die Lösung dann zu einem kleinen Volumen eingedampft und mit Alkohol und Äther versetzt. Es bildete sich ein Niederschlag, der aus reinem Alanin bestand.

0,1539 g der Substanz gaben bei der Verbrennung 0,2274 CO_2 und 0,1070 H_2O .

Für $\text{C}_3\text{H}_2\text{NO}_2$

Berechnet	Gefunden
C = 40,40%	40,29%
H = 7,93%	7,72%

Das Filtrat von Glykokoll und Alanin sollte auf Asparaginsäure und auch möglicherweise auf andere Säuren untersucht werden. Nach der Entfernung der Phosphorwolframsäure wurde eine Probe mit essigsaurem Kupfer gemacht. Beim Eindampfen bildete sich ein Niederschlag, der wohl aus Asparaginsäure bestand, leider aber ging die Hauptlösung verloren.

Auf 100 g berechnet und mit dem Resultat von erhalten, verglichen, bekommt man die folgenden Zahlen:

Glykokoll	}	2,0 g
Alanin		
Aminovaleriansäure	}	17,00 g
Leucin		
Asparaginsäure		?
Glutaminsäure		8,75 g
α -Prolin, inaktives		0,5 g
Tyrosin		1,25%

In manchen Verhältnissen weichen die Zahlen von den von Abderhalden und Pregl¹⁾ erhaltenen ziemlich viel ab, doch kann das auch dadurch verursacht werden, daß wir nicht mit der kristallinischen Substanz arbeiteten.

¹⁾ Zeitschr. f. physiol. Chemie 46, 24, 1905.

Über die Analyse der Spaltungsprodukte des Milz-Nucleoproteids.

Von

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Die Kenntnis der chemischen Natur der Chromatine ist in den jüngsten Jahren durch die Arbeiten mehrerer Forscher bedeutend gefördert worden. Die meisten Arbeiter haben ihr Interesse der Zusammensetzung der Nucleinsäuren gewidmet. Diese Untersuchungen haben aber bisher nur wenige Unterschiede in der Zusammensetzung der Säuren verschiedener Herkunft zutage gebracht. Andererseits haben die Arbeiten von Kossel und seiner Mitarbeiter sehr eklatante Differenzen in den Spaltungsprodukten der Protamine gezeigt. Man dürfte deswegen erwarten, auch bei der Analyse der komplizierteren Nucleoproteide ausgesprochene Unterschiede in der Zusammensetzung des Proteinanteiles zu finden.

Es liegt nur die Analyse eines hierhingehörenden Körpers vor, nämlich die Mitteilung von Wohlgemuth¹⁾ über die Zusammensetzung des Lebernucleoproteids, in welcher eine Reihe sonst im Proteinmolekül unbekannte Körper beschrieben wurde.

Wir haben deswegen beschlossen, eine systematische Untersuchung der Nucleoproteide verschiedener Herkunft vorzunehmen. In der vorliegenden Mitteilung wird über die Resultate der Analyse des Milznucleoproteids berichtet. Die Substanz wurde nach

¹⁾ Zeitschr. f. physiol. Chemie **37**, 475, 1903; **42**, 519, 1904; **44**, 530, 1905. — Ber. d. deutsch. chem. Ges. **37**, 4362.

einem Verfahren, welches schon in einer früheren Arbeit angegeben ist, dargestellt und einmal umgefällt.

Die einzelnen Substanzen wurden auf folgende Weise isoliert:

Aminosäuren.

Glutaminsäure durch Kristallisieren des salzsauren Salzes; Tyrosin durch direktes Kristallisieren. Reines Leucin erhielt man nach dem Verfahren von einem von uns durch Fällen der in Wasser wenig löslichen Aminosäuren mittels Bleizucker und Ammoniak. Aminovaleriansäure wurde erhalten durch Kristallisation der Fraktion aus 75% Essigsäure, die im Filtrate von Blei- und Ammoniakfällung nachgeblieben war. Phenylalanin wurde nur qualitativ nachgewiesen. α -Prolin konnte nicht aufgefunden werden. Asparaginsäure wurde aus der Fraktion der leichtlöslichen Aminosäure mittels Bleizucker und einem großen Überschuß von Ammoniak erhalten. Alanin und Glykokoll wurden mittels konzentrierter Phosphorwolframsäure-ösung und die zweite Säure mittels Picrinsäure gefällt.

Basen.

Alle Basen mit Ausnahme des Thymins wurden mit einer 110-prozentigen Lösung von Phosphorwolframsäure gefällt. In dieser Fraktion wurden die Purin- und Pyrimidin- von den Hexonbasen mittels Mercuriacetatlösung getrennt. Das Thymin wurde aus der Aminosäuren-Fraktion mittels Mercuriacetat gefällt.

Am auffallendsten bei dem Ergebnis der Analyse war der große Gehalt an Glutaminsäure und die Unmöglichkeit, α -Prolin zu gewinnen. Auch Oxyprolin konnte nicht isoliert werden. Die Einzelheiten der Analyse folgen.

Die Darstellung des Nucleoproteids wurde, wie schon bemerkt ist, auf dem früher beschriebenen Wege ausgeführt. Das Präparat wurde einmal umgefällt. 400 g trockne und aschenfrei berechnete Substanz kamen zur Anwendung. Sie wurden in 500 ccm Wasser und 1 l Salzsäure vom spez. Gew. 1,20 aufgenommen und 15 Stunden im Ölbad bei 150° C erhitzt. Die Lösung wurde dann bei vermindertem Druck bis zu 700 ccm eingedampft, in der Kälte mit Salzsäure gesättigt und im Refrigerator bei -1° C mehrere Tage

stehen gelassen. Das auskristallisierte Hydrochlorat der Glutaminsäure wurde auf der Saugpumpe abfiltriert, dann in Wasser gelöst, mit Bariumcarbonat alkalisch gemacht und erhitzt, um das Ammoniak zu verjagen. Das Barium wurde dann quantitativ durch Schwefelsäure entfernt, die Flüssigkeit auf ein kleines Volumen eingedampft und das Hydrochlorat der Glutaminsäure wieder kristallisieren gelassen. Das Salz wurde auf der Saugpumpe abfiltriert, mit Alkohol, welcher in der Kälte mit Salzsäure gesättigt war, gewaschen und bis zum konstanten Gewicht getrocknet. Die Ausbeute betrug 130,0 g.

Die Mutterlauge von dieser Fällung wurde mit der Hauptfraktion vereinigt.

Für die Analyse war ein Teil des Hydrochlorates in die freie Säure übergeführt und diese im Toluolbad getrocknet.

0,2173 g der Substanz gaben 0,3236 CO_2 und 0,1180 g H_2O .

Für $\text{C}_5\text{H}_9\text{NO}_4$

Berechnet	Gefunden
C = 40,79	= 40,61
H = 6,16	6,07

Der Hauptteil der Lösung wurde dann durch wiederholtes Abdampfen bei vermindertem Druck vom Überschusse der Salzsäure befreit und auf etwa 8 l verdünnt, so daß sie etwa einer 5prozentigen Lösung des angewandten Materials entsprach. Diese Lösung wurde dann mit einer 10prozentigen Lösung von Phosphorwolframsäure so lange behandelt, bis sich noch ein Niederschlag bildete. Der Niederschlag, der hauptsächlich Basen enthielt, wurde abfiltriert und die Mutterlauge in üblicher Weise von Phosphorwolframsäure befreit. Die so erhaltene Lösung wurde bis zur beginnenden Kristallisation auf dem Wasserbade eingedampft und erkalten gelassen. Diese Kristallisation bestand hauptsächlich aus Tyrosin und betrug 6,0 g = Fraktion I.

Die Substanz wurde in heißem, verdünntem Ammoniakwasser aufgelöst, die Lösung mit Essigsäure neutralisiert und kristallisieren gelassen. Im Toluolbad getrocknet, wies die Substanz die folgende Zusammensetzung auf:

0,2358 g der Substanz gaben bei der Verbrennung 0,5160 g CO_2 und 0,1286 g H_2O .

Für $C_9H_{11}NO_3$

Berechnet	Gefunden
C = 59,63	= 59,68
H = 6,12	= 6,10

Das Filtrat vom Tyrosin wurde weiter eingedampft und die schwerlösliche Aminosäure auskristallisieren gelassen. Diese Fraktion bestand aus Leucin, Aminovaleriansäure und sehr wenig Phenylalanin, = Fraktion II, und betrug 19,0 g.

Diese Fraktion wurde mit heißem Alkohol ausgekocht, um das möglicherweise anhaftende α -Prolin zu entfernen. Aus dem in heißem absoluten Alkohol unlöslichen Rest wurde dann wieder der in Wasser schwerer lösliche Teil erhalten, in der Hoffnung, reines Leucin zu erzielen. Die Substanz enthielt aber 53,61% C und 9,70% H, war also scheinbar eine Mischung von Aminovaleriansäure und Leucin. Um diese zu trennen, wurde wieder das Verfahren mit Bleizucker und Ammoniak gebraucht. Es wurden nämlich Bleiacetat und Ammoniak zu der konzentrierten Lösung der Aminosäuren zugegeben, solange sich ein Niederschlag bildete. Die Mischung wurde dann erhitzt und heiß filtriert, der Niederschlag in Essigsäure aufgelöst, mit Schwefelwasserstoff vom Blei befreit und die bleifreie Lösung eingedampft, wobei reines Leucin kristallisierte.

0,1515 g der Substanz gaben bei der Verbrennung 0,3035 CO_2 und 0,1376 H_2O .

Für $C_6H_{13}NO_2$

Berechnet	Gefunden
C = 54,96	54,70%
H = 9,92	10,09%

1,0 g der Substanz wurde dann mit Bariumhydrat auf übliche Weise im Autoklaven racemisiert und nach Neuberg und Manasse¹⁾ in α -Naphthylhydantoin übergeführt. Die Substanz hatte den Schmelzpunkt = 169° C.

Das Filtrat von Leucinblei wurde auch von Blei befreit und zu beginnender Kristallisation eingedampft. Es wurde dann stehen gelassen, die erste Fällung wurde nicht untersucht, aber

1) Ber. d. deutsch. chem. Ges. 38, 2359, 1905.

das Filtrat davon wurde zur Trockne eingedampft und aus 80prozentiger Essigsäure umkristallisiert. Die auf diese Weise erhaltene Substanz besaß ohne weiteres Umkristallisieren die Zusammensetzung der Aminovaleriansäure.

0,1515 g im Toluolbad getrocknete Substanz gaben 0,2856 g CO_2 und 0,1278 g H_2O .

Für $\text{C}_5\text{H}_{11}\text{NO}_2$

Berechnet	Gefunden
C = 51,22%	51,41%
H = 9,48%	9,39%

Alle Reste und Filtrate von dieser Fraktion II wurden vereinigt und zur Gewinnung des Phenylalanins verwendet. Zu diesem Zwecke sind sie in Wasser gelöst und durch Eindampfen bei vermindertem Druck möglichst von Essigsäure befreit worden. Die Lösung wurde dann auf ganz kleinem Volumen eingedampft und kristallisieren gelassen. Diese Kristallisation wurde dann durch Oxydieren mittels Kaliumchromat und 25 prozentiger Schwefelsäure auf die Anwesenheit von Phenylalanin untersucht. Man konnte dabei die Bildung von Phenylelessigsäurealdehyd und Benzoesäure wahrnehmen. Es wurde dann versucht, die Säure durch Ausfällen mittels Phosphorwolframsäure zu erhalten. Es gelang aber nicht, die Substanz in reinem Zustande zu gewinnen.

Die Analyse des Filtrates von Fraktion II wurde dann vorgenommen. Diese sollte auf die Anwesenheit von Thymin, α -Prolin, Oxyprolin, Asparaginsäure, Alanin und Glykokoll untersucht werden.

Die Isolierung des Thymins gelang ohne Schwierigkeiten mittels Mercuriacetatlösung. Eine 10 prozentige Lösung des Salzes wurde so lange zugegeben, bis noch ein Niederschlag entstand. Dieser Niederschlag wurde dann auf die übliche Weise vom Quecksilber befreit und die resultierende Lösung bis zur beginnenden Kristallisation eingedampft. Die erste Substanz, die dabei ausfiel, war Tyrosin. Das Filtrat vom Tyrosin wurde weiter eingedampft und kristallisieren gelassen. Die auf diese Weise dargestellte Substanz besaß alle Eigenschaften des Thymins. Zur Analyse war sie aus verdünnter Schwefelsäure umkristallisiert und im Toluolbad getrocknet.

0,1276 g der Substanz gaben bei der Verbrennung 0,2246 g CO_2 und 0,0516 g H_2O .

Für $\text{C}_6\text{H}_6\text{N}_2\text{O}_2$

Berechnet	Gefunden
C = 47,61	48,02
H = 4,76	4,49

Im Filtrate von Thymin war noch eine Substanz mit leicht abspaltbarem Schwefelwasserstoff enthalten. Die Ausbeute an der Substanz war zu klein, um sie rein darzustellen. Es gelang, ein amorphes Silbersalz mit 42,8% Ag und 4,63% S zu gewinnen. Aber auch das Salz reichte nicht für eine vollkommene Analyse.

Die Änderung der Löslichkeit einer Aminosäure bei Anwesenheit von anderen Aminosäuren ist am besten sichtbar am Tyrosin. Es kommt oft bei fraktionierter Kristallisation eine Ausscheidung von Tyrosin vor, nach welcher eine Ausscheidung von anderen Säuren, die vom Tyrosin frei sind, folgt, und dann wieder eine Ausscheidung von Tyrosin.

Das Filtrat vom Mercuriacetatniederschlag war von Quecksilber und möglichst von Essigsäure befreit und die Lösung zur Isolierung der anderen Aminosäuren verwendet; nämlich: α -Prolin, Oxyprolin, Glykokoll, Alanin und Asparaginsäure.

Zu diesem Zweck wurde die Lösung mit einem ganz kleinen Überschuß von Kupferoxyd gekocht, filtriert, bei vermindertem Druck so weit wie möglich eingedampft und dann der Rest in drei Fraktionen geteilt: nämlich in eine in heißem absoluten Alkohol lösliche, in die zweite, die aus dem Reste von der ersten durch Extraktion mittels 75% Alkohol erhalten war, während die dritte aus dem Reste nach der Extraktion der zweiten Fraktion bestand.

α -Prolinfraktion. Die alkohollöslichen Kupfersalze wurden von Kupfer befreit; die Lösung der Fraktion dann zur Trockne eingedampft und mit absolutem Alkohol ausgekocht. Dieser alkoholische Auszug wurde mit dem ähnlichen Auszug der Fraktion II vereinigt, mit der berechneten Menge von Bariumhydrat und Wasser nach Fischers Verfahren bei 150°C im Autoklaven 4 Stunden erhitzt, von Bariumhydrat befreit; die Lösung wieder eingedampft und mit Alkohol extrahiert, und aus diesem Auszuge versucht, das Kupfersalz des inaktiven α -Prolins

darzustellen. Es gelang aber nicht, die Substanz zu gewinnen, obwohl bei der Gelatine und bei dem Eiweißalbumin das ohne Schwierigkeiten möglich war.

Auch aus der Fraktion, löslich in 75% Alkohol, gelang es keine Oxyssäure darzustellen, wie das der Fall bei den zwei anderen Proteinen war.

Der Rest der Kupfersalze wurde von Kupfer befreit, die wässrige Lösung der Fraktion mit Schwefelsäure bis zu einem Gehalt von 5% gebracht und mit konzentrierter Phosphorwolframsäurelösung (4 Teile Säure auf 1 Teil Wasser) behandelt und über Nacht bei -1°C stehen gelassen. Das kristallinische Phosphorwolframat wurde dann auf übliche Weise vom Reagens befreit, bis zu ganz kleinem Volumen eingedampft, mit Alkohol übergossen und über Nacht stehen gelassen. Es bildete sich ein süßschmeckender Niederschlag, der 5,4 g betrug.

Dieser Niederschlag wurde in möglichst wenig Wasser aufgelöst und mit alkoholischer Pikrinsäure behandelt und einige Tage bei -1°C stehen gelassen. Es bildete sich ein kristallinischer Niederschlag, der aus möglichst wenig Wasser umkristallisiert, die Zusammensetzung von Glykokolpikrat ergab: Die Ausbeute betrug 1,6 g.

Die Zusammensetzung des im Toluolbad getrockneten Präparates war die folgende:

0,1601 g der Substanz gaben 24,20 ccm (über 50% KOH) bei $p = 758\text{ mm}$ und $t^{\circ} 28,0^{\circ}\text{C}$.

Für $\text{C}_2\text{H}_5\text{NO}_2 \cdot \text{C}_6\text{H}_2(\text{NO}_2)_3\text{OH}$

Berechnet	Gefunden
N = 18,35%	= 18,38%

Das Filtrat von Glykokolpikrat wurde von Pikrinsäure mittels Äther und Schwefelsäure und dann mittels Baryt quantitativ von Schwefelsäure befreit. Die Lösung wurde dann eingedampft und mit Alkohol behandelt, man erhält auf diese Weise das Alanin.

0,2295 g der Substanz im Toluolbad getrocknet, gaben bei der Verbrennung 0,3372 g CO_2 und 0,1616 g H_2O .

Für $\text{C}_3\text{H}_7\text{NO}_2$

Berechnet	Gefunden
C = 40,41	= 40,07%
H = 7,92	= 7,88%

Das Filtrat vom kristallinischen Phosphorwolframat wurde von Phosphorwolframsäure befreit und zur Darstellung der Asparaginsäure benutzt, es gelang aber nicht, die Substanz rein darzustellen.

Nach dieser Analyse fehlte es also hauptsächlich an α -Prolin und an Asparaginsäure.

Es wurde der Versuch, diese Substanzen darzustellen, wiederholt. 200 g des Nucleoproteids wurden zum Experimente gebraucht. Die Substanz wurde gerade wie beim ersten Versuch hydrolysiert, die Glutaminsäure und die Salzsäure entfernt, und ohne die „Hexonbasen“ zu entfernen, die Tyrosin- und Leucinfractionen abgeschieden. Das Filtrat von der Leucinfraction wurde mit Bleizucker und Ammoniak so lange behandelt, bis sich ein Niederschlag bildete. Es verlangte ziemlich große Quantitäten der Reagenzien. Die Fällung schien sehr voluminös, setzte sich aber zu einem kompakten Niederschlage ab. Dieser wurde dann in Essigsäure aufgenommen, von Blei mittels Schwefelwasserstoff befreit und zu einem kleinen Volumen eingedampft. Die sirupöse Lösung wird mit Alkohol versetzt und stehen gelassen. Es schieden sich dabei lange prismatische Nadeln aus, die stark sauer schmeckten, bei der Sublimation kein Pyrrol lieferten; sie bildeten mit Kupferacetat das typische asparaginsäure Salz und hatten die folgende Zusammensetzung:

0,2768 g der Substanz gaben 0,3696 g CO_2 und 0,1334 g H_2O .

Für $\text{C}_4\text{H}_7\text{NO}_4$

Berechnet	Gefunden
C = 36,07%	36,42%
H = 5,30%	5,39%

Die Ausbeute betrug etwa 1,0 g.

Das Filtrat vom Bleiacetatniederschlage wurde von Blei mittels Schwefelwasserstoff befreit, dann mit Bariumcarbonat gekocht, um das Ammoniak möglichst zu entfernen, das Baryum mit Schwefelsäure entfernt und die Lösung mehreremal bei vermindertem Druck eingedampft, um sie von Essigsäure möglichst zu befreien. Die resultierende Lösung wurde dann mit Kupferoxyd behandelt und gerade wie im vorigen Experimente zur Darstellung des Prolins verwandt. Es gelang aber nicht, die Substanz zu gewinnen.

Basische Bestandteile.

Die „Hexon- und Pyrimidinbasen“ wurden aus demselben Materiale, welches zur Analyse der Aminosäuren angewandt war, dargestellt, während zur Gewinnung der Purinbasen eine besondere Portion von 100 g der trocknen Substanz gebraucht wurde.

Wie schon früher erwähnt, wurden die „Hexon-“ und Pyrimidin-, auch noch der nicht zersetzte Teil der Purinbasen, mittels 10prozentiger Phosphorwolframsäure gefällt. Der Niederschlag wurde in üblicher Weise von der Phosphorwolframsäure befreit, und die Lösung dieser Fraktion mittels einer 10 prozentigen Mercuriacetatlösung in drei Fraktionen geteilt.

Die erste Fraktion enthielt Purinbasen, Cytosin und Histidin. Sie wurde erhalten durch Zusetzen vom Reagens, solange sich noch ein Niederschlag bildete.

Die zweite Fraktion wurde erhalten durch Zusetzen von Barytwasser bis zur alkalischen Reaktion zu dem Filtrate von der ersten Fraktion. Sie bestand hauptsächlich aus Arginin, enthielt aber auch Lysin.

Die dritte Fraktion bestand aus dem Filtrate von Fraktion II und enthielt nur Lysin.

Ich will auch bemerken, daß man aus der Gesamtfraktion noch einige Gramm Leucin gewinnen konnte.

Fraktion I. Die Lösung dieser Fraktion wurde mit Salpetersäure angesäuert, mit einem kleinen Überschuß von Silbernitrat behandelt, filtriert und dann stehen gelassen. Dabei bildete sich ein Niederschlag, der aus Purin-Silbersalzen bestand. Das Filtrat von diesem wurde mit Barytwasser neutralisiert; der sich dabei bildende Niederschlag wurde von Silber und von Baryt befreit, zu einem ganz kleinen Volumen eingedampft und über Schwefelsäure im Exsikkator stehen gelassen. Es bildete sich ein Niederschlag, der aus ganz eigenartigen Kristallen bestand, welche in ihrem Aussehen denen der Pyrimidin- oder Purinbasen nicht ähnlich sahen. Die Ausbeute der Substanz betrug 2,5% und die Zusammensetzung war die folgende:

0,1827 g der Substanz gaben bei der Verbrennung 0,2811 g CO_2 und 0,0725 g H_2O .

Für $C_4H_5N_3O$

Berechnet	Gefunden
C = 43,18%	41,96%
H = 4,53%	4,44%

Die Zusammensetzung also gab die Veranlassung, an Cytosin zu denken. Nun ergab sich, daß die Substanz noch Spuren von Baryt enthielt; wurden diese entfernt, so gelang es ohne Schwierigkeiten, perlmutterglänzende Kristalle des Cytosins zu erhalten. Da sich das Aussehen der Kristalle doch etwas von dem des üblichen Cytosins zu unterscheiden schien, wurde das Präparat mit einem aus Milznucleinsäure dargestellten verglichen. Dasselbe Präparat wurde mit einem von Prof. Wheeler vor einigen Jahren auf synthetischem Wege dargestellten Präparat verglichen. Nun erwies sich der Schmelzpunkt etwas verschieden von dem des synthetischen Cytosins. Das hier erhaltene Präparat sinterte bei $305^{\circ} C$ und zersetzte sich bei 315° .

Das synthetische sinterte bei 310° und zersetzte sich bei $320^{\circ} C$. Nun aber enthielt auch die neue Substanz ein Molekül Kristallwasser.

0,4762 g der lufttrocknen Substanz haben, im Toluolbad getrocknet, 0,0650 g an Gewicht verloren.

Für $C_4H_5N_3O \cdot H_2O$

Berechnet	Gefunden
$H_2O = 13,95$	$= 13,64\%$

Man darf also nicht an das Isocytosin von Wheeler und Johnson denken.

Das Aussehen des Chlorplatinats schien von dem üblichen Chlorplatinat sich etwas zu unterscheiden; das basische schwefelsaure Salz hatte das typische Aussehen des entsprechenden Salzes des üblichen Cytosins. Was die Pikrate betrifft, muß man folgendes bemerken: Wird ein Pikrat aus einem basischen Sulfat eines Cytosinpräparates, welches aus einer Nucleinsäure entsteht, dargestellt, so enthält man gewöhnlich zwei Kristallformen: nämlich lange prismatische Nadeln und andere blätterartige. Bei dem Präparate, welches man in diesem Falle erhielt, wurden alle Kristalle einheitlich und bestanden aus prismatischen Nadeln. Der Schmelzpunkt der Substanz lag bei $265^{\circ} C$.

Es lag also ein Cytosinpräparat vor, welches in den physikalischen Eigenschaften etwas von dem Normalcytosin abwich. Die Ursache der Abweichung kann noch nicht aufgeklärt werden.

Das Filtrat vom Cytosin war mit alkoholischer Picrolonsäurelösung behandelt. Einmal aus ganz wenig Wasser umkristallisiert, besaß die Substanz die Zusammensetzung des Histidinpicrolonats.

0,1483 g der Substanz gaben bei Verbrennung 31,5 ccm Stickstoff (über 50% KOH), bei $t^{\circ} = 23,5$ und $p = 763$ mm.

Für $C_6H_7N_3O_2 \cdot (C_{10}H_8N_4O_5)_2$

Berechnet	Gefunden
N = 23,87%	= 24,02%

Fraktion II. Die Lösung dieser Fraktion wurde nach dem Verfahren von Kutscher und Kossel mit Silbersulfat und Bariumhydrat in drei Fraktionen geteilt. Aus zwei Silberfällungen gelang es mittels Picrolonsäure nur Argininpicrolonat darzustellen. Die Zusammensetzung eines solchen Präparates, einmal aus Wasser umkristallisiert, war die folgende:

0,1590 g der Substanz gaben bei der Verbrennung 35,0 ccm Stickstoff (über 50% KOH), bei $p = 764$ mm und $t^{\circ} = 27^{\circ} C$.

Für $C_6H_{14}N_4O_2 \cdot C_{10}H_8N_4O_5$

Berechnet	Gefunden
N = 25,61	= 25,31

Das Filtrat der Silbersalze wurde von Silber und Baryt befreit und mittels alkoholischer Pikrinsäure behandelt, man erhielt das typische Lysinpikrat.

Aus der Fraktion III gelang es nur Lysin darzustellen. Die Analyse des Pikrates ergab die folgenden Zahlen:

0,1500 g der Substanz gaben bei Verbrennung 0,2113 g CO_2 und 0,0000 g H_2O .

Für $C_6H_{14}N_2O_2 \cdot C_6H_2(NO_2)_3OH$

Berechnet	Gefunden
C = 38,40	38,48%
H = 4,50	4,49%

Die Ausbeute an Histidinpicrolonat betrug etwa 1 g, an Argininpicrolonat 8 g und an Lysinpikrat 29,0 g.

Purinbasen.

Zur Gewinnung dieser Basen wurden 100 g des Proteids mittels 3prozentiger Schwefelsäure 10 Stunden in kochendem Wasserbade erhitzt, die Purinbasen mit Phosphorwolframsäure gefällt und wieder in die Silbersalze übergeführt. Guanin wurde dann als freie Base erhalten und Adenin als Pikrat. Die Ausbeuten betrugen: Guanin = 2,8 g und Adeninpikrat 2,2 g.

Also auf 100 g Substanz berechnet, wurden aus dem Milz-nucleoprotein die folgenden Mengen erhalten:

Glykokol	}	1,5 g
Alanin		
Aminovaleriansäure	}	5,5 g
Leucin		
Phenylalanin		
Asparaginsäure		0,2 g
Glutaminsäure		25,0 g
Tyrosin		1,5 g
Histidin		0,2 g
Arginin		1,0 g
Lysin		3,0 g
Thymin		0,2 g
Cytosin		0,6 g
Adenin		0,8 g
Guanin		2,0 g

Über die chemische Inaktivierung und Regeneration der Komplemente.

Von

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Die Tatsache, daß die Komplementeigenschaften des Serums mit der Zeit von selbst verschwinden, und daß verschiedene physikalische Einwirkungen wie Sonnenlicht und Temperaturen von etwa 55° C, schnell eine Inaktivierung hervorrufen, hat zu der Annahme geführt, daß Komplemente hochgradig labile Verbindungen seien. Es ist jedoch keinerlei Beweis dafür erbracht worden, daß das Verschwinden der genannten Eigenschaft notwendigerweise gleichbedeutend mit einer Molekülzersetzung der fraglichen Substanzen sei. Im Gegenteil ist es durchaus nicht unmöglich, daß eine Inaktivierung hauptsächlich auf Veränderungen des umgebenden Milieus beruht, in welchem die Komplemente ursprünglich wirksam gewesen sind. Bisher hat man der Möglichkeit, daß die übrigen Bestandteile des Serums eine Rolle bei der Art und Weise der Wirkung von Komplementen spielen, wenig Beachtung geschenkt. Dasselbe gilt auch von dem Einfluß gewisser chemischer Substanzen auf die Komplementeigenschaft des Serums.

Gesetzt, man findet einen chemischen Körper, der diese Fähigkeit aufhebt, so ist damit doch noch nicht entschieden, ob er die aktiven Substanzen zerstört oder nur das Milieu in einer Weise ändert, welche die Wirkung der Komplemente beeinträchtigt. Solange wir es mit einer komplizierten Mischung ver-

¹⁾ Vorgetragen auf der Jahresversammlung der Society of American Bacteriologist am 28. Dezember 1906.

schiedener organischer und unorganischer Körper im Serum zu tun haben, können wir keinerlei Schlüsse auf direkte chemische Vorgänge zwischen Komplementen und den hinzugesetzten Substanzen ziehen. Trotzdem wird ein systematisches Studium der Wirkung verschiedener Säuren, Alkalien und Salze auf diese eigentümliche Eigenschaft des Serums ein wertvoller Leitfaden für die Aufklärung der chemischen Struktur der Komplemente sein.

Man kann in jedem Falle hoffen, auf Grund solcher Forschungen bestimmte Folgerungen ableiten zu können. Die vorliegende Arbeit ist unternommen worden, um die Frage der chemischen Stabilität der Komplemente des Serums zu untersuchen. Zu diesem Zweck wurde ihr Verhalten zu verschiedenen Säuren, Alkalien und Salzen geprüft.

Inaktivierung von Komplementen durch Säuren.

Zum Versuch diente Meerschweinchenserum, das als Komplement zur Reaktivierung zweier Arten von Immunamboceptoren gebraucht wurde. Der eine war das auf 56° C erhitzte Serum einer gegen Blutkörperchen vom Rind immunisierten Ziege, und der andere das Serum eines Kaninchens, das mit Hühnerblutkörperchen vorbehandelt war. Die Wirkung der Säuren wurde außerdem an den hämolytischen Bestandteilen des normalen Hundeserums untersucht; in diesem Falle wurden Blutkörperchen vom Kaninchen zur Feststellung der Aktivität nach vorausgegangener Behandlung mit Säuren benutzt.

Die endgültige Mischung von Komplement, Amboceptor, Säure und Blutkörperchen hatte in allen Versuchen ein Volumen von 2,5 ccm, und das osmotische Gleichgewicht wurde durchgehend durch 0,9prozentige Kochsalzlösung hergestellt.

Die Blutkörperchen waren sorgfältig gewaschen und wurden der Flüssigkeit in 5prozentiger Aufschwemmung zugesetzt. Die Beobachtungen wurden nach zweistündigem Stehen im Brutschrank bei 37° C und nach 16stündigem Verweilen bei Zimmertemperatur angestellt.

Die Säurelösungen waren in 0,9prozentiger Salzlösung hergestellt, und jede wurde auf den Säuregrad gebracht, der $\frac{1}{40}$ n-zweibasischer Säure entsprach. Folgende Säuren gelangten zur Verwendung: Salzsäure, Salpetersäure, Schwefelsäure, Phosphorsäure, Ameisensäure, Essigsäure, Propionsäure, Milchsäure, Butter-

säure, Oxybuttersäure, Oxalsäure, Citronensäure, Weinsäure, Fumarsäure, Maleinsäure, Citraconsäure, Itaconsäure, Glycerinphosphorsäure, Harnsäure und Nucleinsäure.

Mit Ausnahme der Harnsäure, die keine hämolytischen Eigenschaften besitzt, entfalteten sämtliche Säuren ungefähr den gleichen Grad von hämolytischer Wirkung, doch waren ausgesprochene Unterschiede in der Schnelligkeit der Reaktion zu bemerken; die Hämolyse erfolgte am schnellsten bei leicht dissoziierbaren Säuren.

In der Regel genügte der Zusatz von 0,4 ccm der Säurelösung ($1/40$ -n) zu 2,5 ccm einer 5prozentigen Aufschwemmung gewaschener Blutkörperchen vom Meerschweinchen gerade, um vollständige Hämolyse zu erzeugen, während 0,2 ccm ohne hämolytische Wirkung blieben.

Die Blutkörperchen des Huhns sind den Säuren gegenüber widerstandsfähiger als die des Meerschweinchens.

Blutserum ist unter normalen Bedingungen mehr oder weniger konstant alkalisch. Zur Prüfung der Wirkung einer Säure auf ihre komplementäre Fähigkeit muß die Änderung in der Reaktion beachtet werden. So wurde die Alkalinität eines angewendeten Serums jedesmal durch Titration festgestellt, bevor Säure hinzugefügt wurde. Auf diese Weise war es möglich, die Beziehungen zwischen komplementärer Wirkung und Reaktion des Serums zu erforschen. Es zeigte sich, daß beim normalen Hundeserum die Alkalinität zwischen $1/45$ und $1/55$ -n bei Prüfung mit einer zweibasischen Säure und Lackmuspapier schwankte. Eine Mischung gleicher Teile von $1/40$ -n Essigsäure und Serum zeigte schwach saure Reaktion.

Die Beziehungen zwischen Reaktion und komplementärer Wirkung des normalen Hundeserums und Meerschweinchenserums sind aus Tabelle I zu ersehen.

Der angegebene Versuch zeigt, daß Komplemente in diesen Serums noch aktiv sind, nachdem die vorhandene Alkalinität durch Neutralisation mit einer Säure beseitigt ist. Er zeigt ferner, daß ein geringes Überwiegen der Säure über die Neutralität hinaus stufenweise eine Verminderung der Wirksamkeit der Komplemente und endlich ihr vollständiges Verschwinden bewirkt. Die Inaktivierung von Komplementärserums durch Säuren ist von dem Verbrauch einer gewissen Menge von Säure begleitet. $4/10$ ccm von $1/40$ -n-

Essigsäure sind mehr als hinreichend, um die Blutkörperchen in obiger Mischung zu lösen, wenn gleichzeitig kein Serum vorhanden ist. Es sieht so aus, als sei die Inaktivierung des Komplements eng mit dem Verschwinden von Säure verknüpft. Wenn Säure von den Proteinen des Serums aufgenommen worden ist, so wird ihre Anwesenheit durch die Blutkörperchen festgestellt. Dieses gleichzeitige Unwirksamwerden von Säure und Komplement ist vielleicht die Folge der Entstehung einer unwirksamen Verbindung dieser beiden Stoffe. Man mag gegenüber dieser Annahme deswegen Einwände erheben, weil das 30 Minuten lang auf 56° C erhitzte Serum ebenso antihämolysisch gegen Säuren wirkt, aber dieser Einwand ist unbegründet, da die Inaktivierung des Serums bei dieser Temperatur nicht auf Zerstörung des Komplements zu beruhen braucht.

Tabelle I.

Hundeserum 0,3 ccm			Meerschweinchen Serum 0,3 ccm (Komplement)		
Menge der Essigsäure $\frac{1}{40}$ -n	Reaktion der Mischung	Hämolysische Wirkung der Mischung auf 25 ccm 5proz Meer- schweinchen-Blut- körperchen	Reaktion der Mischung	Hämolysische Wirkung des Gemisches + 0,1 ccm Antischäfer Serum pro Roh- chen auf 25 ccm 5proz Schaffblutkörperchen	
0,6	sauer	mäßige Hämolys	sauer	mäßige Hämolys	
0,5	"	leichte Hämolys	"	keine Hämolys	
0,4	"	keine Hämolys	"	" "	
0,3	schwach sauer	mäßige Hämolys	neutral	c. Hämolys (verzög.)	
0,25	schw.alkalisch	c Hämolys (verzög.)	schw alkalisch	" "	
0,2	alkalisch	" " "	mäßig " "	" "	
0,15	"	" " "	alkalisch	" "	"
0,1	"	" " "	"	" "	
0,07	"	" " "	"	" "	
0,05	"	" " "	"	" "	"
0	"	" " "	"	" "	"

Wenn wir als Erklärung die fermentartige Natur des Komplements annehmen, so würde der Wechsel in der Reaktion durch überschüssige Säure eine Deutung für die Inaktivierung des Serums abgeben. Aber eine derartige Annahme sollte möglichst so lange vermieden werden, als die geringste Hoffnung besteht, die Erscheinung auf einfachere Weise zu erklären. Wärmeunbeständigkeit und andere Eigenschaften, die allen sogenannten

Fermenten gemeinsam sind, müssen insoweit aufgegeben werden, als sie die Labilität dieser Körper betreffen, und schärfere Beobachtungen sollte man über die Rolle jener Stoffe anstellen, welche zusammen mit den wirklichen aktiven Bestandteilen in dem Ausgangsmaterial vorhanden sind.

Die inaktivierende Wirkung stellte sich als gemeinsame Eigenschaft der Säuren heraus, wenn auch von Phosphorsäure sowie von Glycerinphosphorsäure verhältnismäßig größere Dosen als von den anderen verbraucht wurden. Harnsäure besitzt nur schwach hemmende Kraft.

Inaktivierung von Komplementen durch Alkalien.

Die Methode war im wesentlichen dieselbe wie bei den Experimenten mit Säuren. Verwendet wurden die Hydroxyde von Ammonium, Natrium, Magnesium, Calcium und Barium in $\frac{1}{40}$ n-Lösung.

Die Lösungen wurden in 0,9 prozentiger Salzlösung bereitet.

Die hämolytische Kraft dieser Alkalien war ungefähr die gleiche, abgesehen davon, daß die Wirkung der verschiedenen Alkalien mit verschiedener Schnelligkeit eintrat. $\frac{5}{10}$ ccm der $\frac{1}{40}$ n-Lösung aller Alkalien außer Ammoniak bewirkten vollständige Hämolyse innerhalb 2 Stunden, während dieselbe Wirkung durch 1 ccm Ammoniaklösung erfolgte. Die Blutkörperchen der Schafe zeigten gegenüber Alkalien größere Widerstandskraft und erforderten 0,7 ccm zur vollständigen Hämolyse. Nachdem die hämolytische Kraft dieser Alkalien geprüft worden war, wurde ihre Wirkung auf die komplementäre Eigenschaft von Hundeserum und Meerschweinchenserum untersucht. Die Mischung wurde während 15 Minuten bei 37° C gehalten, dann wurden Blutkörperchen oder Blutkörperchen plus Amboceptoren hinzugefügt.

Aus Tabelle II ist zu ersehen, daß durch Hinzufügung von geeigneten Mengen verschiedenartiger Alkalien die komplementäre Eigenschaft des Serums verloren geht oder an Stärke einbüßt. Ammoniumhydroxyd bewirkte die sicherste, Calciumhydrat dagegen die geringste Hemmung. Natronlauge wirkt für eine bestimmte Zeit hemmend, nach deren Verlauf schließlich Hämolyse erfolgt. Es muß bemerkt werden, daß die hämolytische Wirkung dieser Alkalien gleichzeitig mit der komplementären Eigenschaft verschwindet. Wie bereits gezeigt, werden die Komplemente durch

Säuren inaktiviert, und nun sehen wir, daß Alkalien auch inaktivierend wirken. Nehmen wir an, Komplemente seien Fermente, so läßt sich das Phänomen der Inaktivierung ohne Schwierigkeit erklären, indem wir diese einer ungünstigen Reaktion zuschreiben können, die im Milieu durch die Chemikalien hervorgerufen wird. Tatsächlich ist gegen die Annahme, daß Komplemente salzartige Körper seien, in denen der basische oder saure Rest durch verschiedenartige Säuren oder Alkalien ersetzt werden könnte, nichts einzuwenden. Eine solche Umsetzung mag wirklich die Ursache der Inaktivierung sein. Daß die Hemmung der komplementären Wirkung durch verschiedenartige Säuren oder Alkalien nicht der Zunahme der Salzkonzentration in der Flüssigkeit oder der Bildung gewisser Verbindungen zuzuschreiben ist, die sich aus der Wechselwirkung zwischen den eingeführten Säuren und den vorhandenen Alkalien ergibt, ist durch entsprechende Experimente bewiesen worden.

Tabelle II.

Menge der alkalischen Flüssigkeit	Hundeserum 0,5 cem			Meerschweinchenserum 0,3 cem (Komplement)		
	Hämolytische Wirkung der Mischung auf 2,5 cem 5proz. Meerschweinchen-Blut- körperchen			Hämolytische Wirkung des Gemisches + 0,1 cem Antischäferum pro Röhrchen auf 2,5 cem 5proz. Schafblutkörperchen		
	$\frac{1}{40}$ n-Ammoniak	$\frac{1}{40}$ n-Natron-lauge	$\frac{1}{50}$ n-Calcium-hydrat	$\frac{1}{40}$ n-Ammoniak	$\frac{1}{40}$ n-Natron-lauge	$\frac{1}{50}$ n-Calcium-hydrat
1	keine Häm.	c. Hämolyse	c. Hämolyse	keine Häm.	c. Hämolyse	c. Hämolyse
0,8	" "	" "	" "	" "	" "	" "
0,7	" "	starke Häm.	starke Häm.	" "	" "	" "
0,6	" "	schw. Häm.	" "	" "	" "	" "
0,5	mäßige Häm.	keine Häm.	mäßige Häm.	" "	schw. Häm.	starke Häm.
0,4	c. Hämolyse	" "	schw. Häm.	c. Hämolyse	keine Häm.	" "
0,35	" "	" "	" "	" "	schw. Häm.	schw. Häm.
0,25	" "	schw. Häm.	starke Häm.	" "	mäßige Häm.	starke Häm.
0,2	" "	c. Hämolyse	c. Hämolyse	" "	c. Hämolyse	c. Hämolyse
0,15	" "	" "	" "	" "	" "	" "
0,1	" "	" "	" "	" "	" "	" "
0	" "	" "	" "	" "	" "	" "

Der nächste Schritt war, Gewißheit darüber zu erlangen, ob bestimmte Salze die komplementäre Wirkung des Serums hemmen. Das war wegen der gegebenen Möglichkeit wichtig, die engeren Beziehungen zwischen der Inaktivierung von Komplementen und

der chemischen Struktur gewisser antikomplementärer Substanzen zu studieren. Bei Annahme, daß die Komplemente von salzartiger Natur sind, müßten wir natürlich erwarten, daß gewisse Salze auf Komplemente einwirken und andere nicht, gemäß der Affinität der diese Salze zusammensetzenden Radikale. Eine diesbezügliche Untersuchung ist darum für die Erforschung der chemischen Struktur der Komplemente von großer Bedeutung.

Inaktivierung der Komplemente durch Salze.

Daß verschiedene Elektrolyte die Komplemente unwirksam machen können, ist durch Hektoen¹⁾ und Manwaring²⁾ gezeigt worden. Beide fassen die Erscheinung als einen chemischen Vorgang auf. Über die Wirkung von Säuren und Alkalien haben sie keine systematische Untersuchung angestellt, obgleich sie den Kationen eine wichtige Rolle bei der Inaktivierung von Komplementen zuschreiben. Während die Wirkung der Säuren und Alkalien keine bestimmte Beeinflussung der alkalischen oder sauren Komponente der Komplemente zu erkennen gibt, dürfte die Reaktion zwischen verschiedenen Salzen und Komplementen einen ziemlich genauen Anhalt abgeben, nach dem die Affinität des betreffenden Radikals in letzterem berechnet werden kann.

Die Versuche mit Säuren haben uns schon gelehrt, daß Harnsäure nicht imstande ist, das hypothetische Säureradikal der Komplemente zu substituieren, während die andern Säuren, die meisten mineralischen und organischen Säuren mit einbegriffen, die Komplemente zu spalten und inaktive Körper zu bilden scheinen.

Die meisten freien Alkalien, besonders Natronlauge, substituieren das hypothetische Basenradikal des Komplements und führen zur Bildung entweder einer schwächeren oder beinahe unwirksamen Verbindung oder einer Verbindung, deren Aktivität in einem Serumproteine enthaltenden Medium aufgehoben ist.

A. Wirkung von Salzen, die aus starker Base und starker Säure zusammengesetzt sind.

Chloride, Sulfate oder Nitrate von Kalium und Natrium sind nicht antikomplementär, wenn nicht die Konzentration

1) Hektoen, Trans. of the Chicago Path. Society 5, 303, 1903.

2) Manwaring, Journ. of Infections diseases 1, 112, 1904.

stärker als einfach normal ist, in welchem Falle die Hämolyse gänzlich oder teilweise aufgehoben werden kann. Die Wirkung ist eine physikalische und keine chemische. Daraus kann man schließen, daß der saure oder basische Rest der Komplemente zu schwach ist, einen der Bestandteile des erwähnten Salzes abzuspalten. Bei Natriumsulfat läßt sich mehr oder weniger eine Hemmung in der Hämolyse beobachten.

B. Wirkung von Salzen, die aus schwacher Base und starker Säure bestehen.

Von den unter diese Rubrik gehörenden Substanzen untersuchte ich die Chloride, Nitrate, Sulfate, Phosphate, Acetate, Oxalate und Citrate von Ammonium, Neurin, Magnesium, Calcium und Barium. Die Ca- und Ba-Salze bewirkten, unabhängig von ihrer Löslichkeit, vollständige Inaktivierung der Komplemente, wenn sie in einer Konzentration von $\frac{1}{20}$ -n zur Verwendung kamen. Magnesiumsalze wirken mehr oder weniger hemmend, doch kann Hämolyse nach mehrstündigem Verweilen im Brutschrank erfolgen. Ammonium- und Neurinsalze besitzen keine inaktivierenden Eigenschaften.

Hier haben wir einige neue Tatsachen gewonnen. Es findet eine chemische Wechselwirkung zwischen Komplementen und einigen Erdalkalisalzen starker Säuren statt, und keine Umsetzung erfolgt mit Ammonium- und Neurinsalzen. Der Grund dieser Verschiedenheiten kann in der Tatsache gesucht werden, daß das basische Radikal des Komplements ungefähr dieselbe Affinität wie Ammonium oder Neurin besitzt, jedoch vielleicht eine stärkere gegenüber starken mineralischen oder organischen Säuren als gegenüber dem eigenen Säureradikal. Als Ausdruck dieser Verschiedenheit der Affinitäten würde die Bildung eines Calcium- oder Bariumsalzes des Komplementen und die Bildung eines Salzes zwischen einer starken Säure und dem basischen Rest des Komplements erfolgen. In diesem Falle sind die Produkte nicht imstande, Amboceptoren zu aktivieren, daher tritt auch keine Hämolyse ein. Diese Erscheinung läßt sich leicht durch zwei chemisch charakterisierte Körper hervorrufen. Wenn Natriumcarbonat mit Calciumchlorid gemischt wird, tritt Bildung von unlöslichem Calciumcarbonat und Chlor-

natrium ein, und die vorhandene hämolytische Kraft der Soda wird aus der Mischung verschwinden.

C. Wirkung von Salzen, die aus starker Säure und schwacher Base bestehen.

Als schwache Säuren bezeichnet man die höheren Fettsäuren und die Kohlensäure. Das Carbonat und Bicarbonat des Natriums kommen hier in erster Reihe in Betracht. Natriumsalze von Stearin-, Palmitin- und Ölsäure sind hämolytisch befunden worden und ihre Wirkung auf Komplemente ist eine beschleunigende anstatt hemmende. Auf diese Besonderheit werde ich an anderer Stelle zurückkommen.

Soda ist an sich hämolytisch und verursacht in ganz minimalen Dosen nur leichte Hemmung. Natriumbicarbonat wirkt ausgesprochen inaktivierend, vielleicht weil es ein Hydroxylion im Molekül besitzt. Tabelle III zeigt das Resultat.

Tabelle III.

Menge der	2,5 ccm 5proz. Aufschwemmung von Meerschweinchen-Blutkörperchen			
	Plus $\frac{1}{40}$ n-Natriumcarbonatlösung	Plus $\frac{1}{40}$ n-Natriumcarbonatlösung und Hundeserum 0,5 ccm	Plus $\frac{1}{40}$ n-Natriumbicarbonatlösung	Plus $\frac{1}{40}$ n-Natriumbicarbonatlösung und Hundeserum 0,5 ccm
1	keine Häm.	Teilweise Häm.	keine Hämolyse	Partielle Hämol.
0,7		c. Häm.(st.verz.)		c. Häm. (st. verz.)
0,5		c. H.(schw.verz.)		" " " "
0,3		" " " "		c. H. (schw.verz.)
0,2		" " " "		" " " "
0		" " " "		" " " "

D. Wirkung von Salzen zwischen schwacher Base und schwacher Säure.

Ich prüfte das Carbonat, Stearat und Oleat von Ammonium, Neurin, Magnesium, Calcium und Barium. Von diesen waren alle Carbonate von alkalischen Erden und des Ammoniums in $\frac{1}{10}$ n-Lösung (in 0,9% NaCl) gebracht, die Seifen hingegen in der Regel in $\frac{1}{100}$ n-Lösungen.

Die Resultate waren eindeutig insofern, als alle Carbonate, die in Mengen von 2 ccm (bei 2,5 ccm Totalvolumen) angewandt

waren, die Hämolyse nicht deutlich zu hemmen vermochten. Andererseits wirkten verschiedene Seifen ungleichartig. Kurz, alle Erdalkaliseifen, ausgenommen Magnesiumseife, sind indifferent gegenüber der Einwirkung von Komplementen, während Magnesiumseife eine leicht beschleunigende Wirkung ausübt. Die Inaktivität der Calcium- und Bariumseifen mag ihrer Unlöslichkeit in dem salzhaltigen Medium zugeschrieben werden. Gewisse lösliche Seifen, z. B. Natrium-, Ammonium- und Neurinoleat, haben keine antihämolytische Eigenschaft in Mengen, die selber nicht mehr hämolytisch sind.

Sie erhöhen im Gegenteil die komplementäre Eigenschaft eines gegebenen Serums. Bemerkt sei, daß die löslichen Ölseifen in hohem Maße in einer proteinfreien Flüssigkeit hämolytisch sind. Die Erklärung für diese Erhöhung der komplementären Kraft des Serums bei Hinzufügung von löslichen Ölseifen ist in einer besonderen Abhandlung¹⁾ gegeben worden.

Regeneration von Komplementen, die einmal durch Chemikalien inaktiviert waren.

Die wahrscheinlich chemische Natur und die Stabilität der Komplemente ist durch die vorangegangenen Versuche dargetan. Jetzt bleibt noch zu untersuchen, ob sie nach Inaktivierung der komplementären Substanzen mittels gewisser Säuren, Alkalien und Salzen wieder aktiv gemacht werden können. Wo es sich um Säuren oder Alkalien handelt, würde einfache Neutralisation zu diesem Zweck genügen, doch bei Salzen ist eine Umkehrung der Reaktion notwendig, vorausgesetzt, daß die hier über die Struktur der Komplemente vertretene Ansicht zutreffend ist.

A. Regeneration der durch Säure inaktivierten Komplemente.

Es ergab sich, daß 0,6 ccm einer $1/40$ n-Lösung einer einbasischen Säure, 0,4 ccm einer zweibasischen, 0,3 ccm einer dreibasischen und 0,2 ccm einer vierbasischen Säure, ausgenommen gewisse höhere Fett-, Acryl- oder Carbonsäuren, innerhalb einer

¹⁾ H. Noguchi, On certain chemical complementary Substances, Proc. of the Soc. of Exper. biology and Medicine New York 4, 45, 1907.

halben Stunde bei 37°C eine Inaktivierung von 0,5 ccm frischen wirksamen Hundeserums hervorrufen. HCl , HNO_3 , H_2SO_4 , H_3PO_4 , Essig-, Propion-, Milch-, Butter-, Oxybutter-, Oxal-, Wein-, Citronen-, Malein- und Citraconsäure ergaben alle dasselbe Resultat. Um zu sehen, ob die durch diese Säuren inaktiv gemachten Komplemente wieder zur Aktivität gebracht werden können, wurden die Säuren genau mit Natronlauge neutralisiert. Das Serum ließ man nach der Neutralisation 15 Minuten bei 37°C stehen und fügte dann eine 5prozentige Aufschwemmung von Schafs- oder Meerschweinchenblutkörperchen hinzu. Als Ergebnis zeigte sich, daß diejenigen Komplemente, die zuvor durch Propion-, Malein-, Wein- und Citraconsäuren inaktiviert worden waren, gleich nach der Neutralisation ihre ursprüngliche Aktivität wieder erlangen. Das mit Essigsäure, Oxalsäure und Buttersäuren behandelte Serum erlangt sie weniger vollständig zurück, als dies bei den zuerst erwähnten Säuren der Fall war. Die durch mineralische Säuren hervorgerufene Inaktivierung ist ausgezeichnet durch die langsame Rückkehr der komplementären Wirkung nach Neutralisation dieser Säuren durch Alkali.

Ammoniak ist gleichfalls imstande, die Wirkung der Komplemente wieder herzustellen, doch nicht in so vollkommenem Maße wie Natronlauge. Alle Mineralsäuren, die stärker als einfach normal sind, führen dauernde Inaktivität der Komplemente herbei.

B. Regeneration der durch Alkalien inaktivierten Komplemente.

Im allgemeinen gelingt die Regeneration eines Komplements, das durch Alkalien inaktiviert wurde, schwieriger und weniger sicher, als wenn es mit Säuren behandelt worden war. Die vollkommenste Wiederherstellung erzielte man, wenn die Alkalien mit Essig- oder Propionsäure neutralisiert wurden. Salzsäure gab oft negative Resultate.

C. Regeneration von Komplementen, die durch Salze inaktiviert waren.

Manwaring¹⁾ hat schon beobachtet, daß Komplemente, die durch gewisse Salze inaktiviert wurden, dadurch, daß man

¹⁾ a. a. O.

sie vom Fällungsmittel befreite, wieder aktiv gemacht werden können.

Bei meiner Untersuchung fand ich, daß das durch Ca oder Ba (in Form von Chlorid, Nitrat, Sulfat oder Acetat) inaktivierte Serum seine komplementäre Eigenschaft wieder erlangte, wenn eine genügende Menge Natriumtarbonat hinzugefügt wurde. Der Grad der Aktivität der so behandelten Komplemente wird immer unterhalb der ursprünglichen Höhe gefunden. Das teilweise regenerierte Serum wird durch Erwärmung auf 56° C wieder inaktiv gemacht.

Zusammenfassung.

Jede Säure, die stärkere Affinität als Kohlensäure und die höheren Fett- oder Acrylsäuren besitzt, inaktiviert Komplemente in einer Konzentration von ungefähr $1/40$ -n bei zweibasischen Säuren, wenn diese einer gleichen Menge Serum zugesetzt wird. In gleichem Grade, wie die Wertigkeit der Säure zunimmt, verringert sich die erforderliche Menge. Kohlensäure sowie einige höhere Fettsäuren bewirken keine Inaktivierung.

Verschiedene Alkalien inaktivieren Komplemente, wenn sie in einer Konzentration von ungefähr $1/60$ — $1/40$ -n vorhanden sind, wechselnd mit der Natur des verwendeten Alkalis. Natronlauge wirkt schnell, doch ist die Hemmung, die sie verursacht, weniger ausgesprochen als die durch Ammoniak, von dem eine größere Menge als von ersterer nötig ist. Calciumhydroxyd zeigt die geringste Wirkung.

Salze von starken Säuren und starken Basen wirken nicht hemmend, außer wenn ihre Konzentration $1/1$ -n nahezu erreicht; in $1/8$ n-Lösung wirken sie auf ein Komplement nicht inaktivierend. Salze starker Säuren und schwacher Basen und Salze schwacher Säuren und starker Basen inaktivieren in $1/10$ -n oder sogar in schwächeren Lösungen. Ca- und Ba-Salze haben die stärkste hemmende Wirkung auf Komplemente. Salze von schwachen Säuren und schwachen Basen besitzen keine inaktivierende Eigenschaft. Gewisse lösliche Ölseifen, welche an sich in einem eiweißfreien Medium erheblich hämolytisch sind, erhöhen die Wirkung von Komplementen bedeutend, wenn sie in ganz minimalen Dosen dem Serum zugefügt werden.

Die Wirkung von Komplementen, die durch Hinzufügung entsprechender Mengen von Säuren, Alkalien und Salzen aufgehoben oder vermindert worden ist, kann gänzlich oder teilweise durch Entfernung dieser Zusätze mittels Neutralisation oder Fällung wieder hergestellt werden.

Die regenerierten Komplemente können so aktiv wie vorher oder auch weit weniger wirksam als ursprünglich sein. Mineralsäuren neigen zur Erzeugung dauernder Unwirksamkeit, wenn sie in einer ca. $\frac{1}{1}$ n-Lösung angewandt werden. Die regenerierten Komplemente werden inaktiv bei Erwärmung auf 56°C , wenn sie eine halbe Stunde andauert. Die Komplemente sind hinsichtlich des Einflusses der Reaktion des Milieus auf ihre Wirkungsweise gewissen Fermenten zu vergleichen. Und doch scheinen sie besser mit aus schwacher Säure und schwacher Base bestehenden Salzen als mit den Fermenten in Parallele gestellt zu werden. Das sind einige, wenn auch nicht ausreichende Gründe für die Annahme, daß Komplemente Salze der Ölsäure oder höherer Fettsäuren mit organischen Basen sein könnten.

Über eine lipolytische Form der Hämolyse.¹⁾

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Die lipolytische Form der Hämolyse ist besonders wegen ihrer biologischen Stellung unter den verschiedenen Arten der Hämolyse von Interesse. Säuren, Alkalien, gewisse Salze und Glucoside zerstören die Blutkörperchen direkt und sind unfähig, auch bei wiederholter Injektion Antikörperbildung im Organismus anzuregen. Taurocholsaures Natrium sowie cholsaures Natrium scheinen jedoch eine Ausnahme zu bilden, da Rist und Ribadeau-Dumas²⁾ sowie Nicolle³⁾ eine Zunahme der antihämolytischen und antitoxischen Eigenschaften des Serums von Tieren beobachteten, die mit jenen Salzen vorbehandelt war. Verschiedene Hämolysine bakterieller Herkunft zerstören die Zellen in ähnlicher Weise, aber die eigentliche Natur des Lösungsvorganges ist unbekannt. Einige von ihnen sind hitzebeständig und vermutlich von ziemlich einfacher Struktur, während ein anderer Teil mehr den Fermenten ähnelt, insofern sie termolabil, mit der Zeit leicht zerstörbar und im trockenen Zustande beständig sind. Diesen nahe steht eine andere Klasse von Hämolysinen, die allein in Gegenwart einer besonderen zweiten Komponente wirksam sind, welch letztere

1) Read before the Soc. for Exper. Biol. and Med., 1907, May 20, New York.

2) Rist et Ribadeau-Dumas, Essai d'immunisation du lapin contre l'action hémolytique du taurocholate de soude. Compt. rend. d. l. Soc. d. Biol. 55, 1519, 1903.

3) Nicolle, Séro-immunité vis-à-vis du „Choléate de soude“. Annales de l'Inst. Pasteur 21, 26, 1907.

allein inaktiv ist. Diese komplexe Gruppe umfaßt die Serumhämolsine und die Schlangengifthämolsine. Die Wirkung dieser komplexen Hämolsine ist selektiv oder spezifisch, namentlich dann, wenn das Serum auf immunisatorischem Wege bereitet ist. Die Spezifität beruht auf der Gegenwart der sogenannten Zwischenkörper oder sensibilisierenden Substanzen, die eine größere Resistenz gegen Hitze haben als die anderen nicht spezifischen Komponenten, die sogenannten Komplemente oder Alexine. Den Zwischenkörpern, besonders Paul Ehrlichs Amboceptoren, entspricht das Auftreten spezifischer Antikörper bei Einführung in einen fremden Organismus, obgleich die Bildung der Antikomplemente im Tierkörper streng genommen eine Hypothese ist. Auf alle Fälle macht die Bildung spezifischer Präcipitine im Immunsérum den absolut sicheren Beweis für die Existenz der Antikomplemente unmöglich. Die chemische Natur der Amboceptoren oder Immunkörper ist noch unbekannt. Wir wissen nur, daß sie durch Erhitzung auf 75°C oder höher ihre Wirksamkeit verlieren, während die Giftamboceptoren sogar bei Siedetemperatur beständig sind. L. von Liebermann¹⁾ hat in einer jüngst erschienenen, nur wenig später als eine Mitteilung ähnlichen Inhalts von Noguchi²⁾ veröffentlichten Arbeit über Komplemente gezeigt, daß Ölsäure in Gegenwart bestimmter Seifen als Immunkörper fungieren kann. Bezüglich der Komplemente meint Noguchi (l. c.), daß bestimmte lösliche Seifen, namentlich Ölseifen, unter bestimmten Bedingungen alle wesentlichen Eigenschaften der Komplemente erlangen. Er gibt jedoch zu, daß gewisse Unterschiede zwischen den natürlichen und künstlichen Komplementen bestehen. Kyes' allbekannte Arbeiten über die Bildung der Giftlecithide, denen sich Morgenroths und Karpis³⁾ Untersuchung über das Toxolecithid des Bienengiftes anschließt, verbreiteten etwas Licht über die hämolytischen Vorgänge bei diesen Giften. Während Kyes annimmt, daß das Lecithin im Blut geradezu für die Gifthämolyse verantwortlich

¹⁾ v. Liebermann, Über Hämoagglutination und Hämatolyse. Diese Zeitschr. 4, 25, 1907.

²⁾ Noguchi, On certain chemical complementary substances. Proc. of Soc. Exper. Biol. and Med. 4, 45, 1907.

³⁾ Morgenroth und Carpi, Über ein Toxolecithid des Bienengiftes. Berl. klin. Wochenschr. 43, 1424, 1906.

zu machen ist, betont Noguchi¹⁾, daß gewisse Fettsäuren und lösliche Seifen für die Hämolyse mehr in Betracht kommen als das Lecithin unter gewöhnlichen Verhältnissen. Von großem Interesse ist die Entdeckung Neuberg und Rosenberg's²⁾ vom Vorkommen eines ausgesprochenen fettspaltenden Agens in verschiedenen Giften und Phytotoxinen, obgleich noch keine bestimmte Beziehung zwischen hämolytischer und lipolytischer Fähigkeit selbst hergestellt ist. Neuberg und Reicher³⁾ fanden, daß gewisse antibakterielle Immunsera ein größeres Fettspaltungsvermögen als Normalsera der entsprechenden Spezies besitzen. Die Untersuchungen von Neuberg, Rosenberg und Reicher machen es in hohem Grade wahrscheinlich, daß gewisse Beziehungen zwischen Lipolyse und Hämolyse (und sogar zwischen Bakteriolyse) bei bestimmten Substanzen oder Seris bestehen. Längere Zeit vor dem Erscheinen dieser Arbeiten habe ich über die Bedeutung der Hämolyse als eine Folge von Lipolyse gearbeitet. Vor mehreren Jahren hatte ich beobachtet, daß Ölsäure zehnmal stärker hämolytisch wirkt als gewöhnliche Mineralsäuren oder normale Fettsäuren⁴⁾, und ich dachte an die Möglichkeit einer lipolytischen Form der Hämolyse. Da ich jedoch ein altes fettspaltendes Ferment (Steapsin Kahlbaum) benutzt hatte, erhielt ich keine Lipolyse. Jetzt wendete ich meine Aufmerksamkeit der Pankreaslipase zu und erhielt nun ein positives Resultat, dessen Wiedergabe den Gegenstand der vorliegenden Mitteilung bildet.

In der Lipase habe ich nun eine weitere Form eines komplexen Hämolsins gefunden. Wenn man die Lipase durch Alkohol-fällung einer rohen Pankreasemulsion darstellt und sie dann durch Ätherextraktion von Fett befreit, so ist sie hämolytisch unwirksam. Wenn man sie aber dann mit einem an sich nicht hämolytischen höheren Neutralfett zusammenbringt, so tritt vollständige Hämolyse ein. Lipolyse und Hämolyse gehen Hand in Hand miteinander.

1) Noguchi, On extracellular and intracellular venom activators of the blood, with especial reference to lecithin and fatty acids and their compounds. Journ. of exper. Med. 9, 436, 1907.

2) Neuberg und Rosenberg, Lipolyse, Agglutination und Hämolyse I. Berl. klin. Wochenschr. 44, 54, 1907.

3) Neuberg und Reicher, Lipolyse, Agglutination und Hämolyse II. Diese Zeitschr. 4, 281, 1907.

4) Noguchi, On certain thermostabile venom activators. Journ. of exper. Med. 8, 87, 1906.

In diesem Stadium ist die Hämolyse eine direkte Folge der Fettspaltung und auf die Wirkung freier Fettsäuren oder ihrer Verbindungen zurückzuführen.


Im folgenden möge eine kurze Wiedergabe meiner Versuche folgen.

Darstellung von Lipase.

Erste Methode. Frisches Hunde- oder Meerschweinchenpankreas wurde mit dem 20fachen Gewicht Wasser digeriert. Es wurde sodann koliert und mit dem mehrfachen Volumen Alkohol von 95% gefällt. Nach mehreren Stunden wurde der Niederschlag abfiltriert und auf einem Filter gesammelt, sodann mit Äther erschöpft und getrocknet.

Zweite Methode. Die Pankreasemulsion wurde in gleicher Weise wie bei Methode 1 hergestellt, dann aber mit gesättigter Uranylacetatlösung (20 : 100) gefällt. Der Niederschlag wurde auf einem Filter gesammelt, getrocknet und pulverisiert. Das Pulver wurde sorgfältig mit Äther extrahiert und von jeder Spur anhaftender Fettsubstanzen befreit. Das Pulver wurde dann vom anhaftenden Äther befreit und für die folgenden Versuche benutzt.

Neutralfette für die Lipolyse.

 Ich prüfte die Wirkung meiner Lipase auf Äthylformiat, Äthylbutyrat, auf Butter, Akrolein, Tributyrin, Crotonöl, Trolein, Tripalmitin, Tristearin und auf die durch Ätherextraktion aus dem Fettgewebe und Mesenterium von Hund und Meerschwein gewonnenen Fette. Angesichts der anhaftenden hämolytischen Fähigkeit oder wegen der vollkommenen Unlöslichkeit wurden die meisten Ester, niederen Glyceride und das unlösliche Palmitin und Stearin für die scharfe Probe der Hämolyse als ungeeignet befunden.

Befriedigende Resultate erhält man mit Triolein, Butter und den Fettgemischen tierischer Herkunft.

Blutkörperchen für die Hämolyse.

Zur Untersuchung dienten gewaschene Blutkörperchen von Hund oder Meerschwein, und zwar in einer 5prozentigen Aufschwemmung.

Ausführung.

Das gesamte Volumen der Mischung, das die nötige Menge der Komponenten für den Versuch enthielt, betrug 2 ccm. Die Isotonie

wurde durch 0,9prozentige Kochsalzlösung hergestellt. Die Einwirkungszeit betrug 4 Stunden bei 37° C, worauf der Umfang der Lipolyse oder Hämolyse festgestellt wurde.

Versuchsergebnisse.

Wenn man einen Tropfen Tirolein, tierischen Fettes oder geschmolzener Butter zu einer Suspension gewaschener Blutkörperchen eines der erwähnten Tiere bei Gegenwart einer ausreichenden Menge Lipase fügt — 1 ccm 5prozentiger Emulsion des Pankreaspulvers —, so tritt vollständige Hämolyse ein. Weder Lipase noch Fett allein bringen sie hervor.

Lecithin kann in diesen Neutralfetten nicht vorhanden sein. So weit meine bisherigen Experimente reichen, zeigt sich, daß der Alkohol-Pankreasniederschlag Lecithin nicht in nennenswertem Maße spaltet. Ob bei Darstellung ohne Alkohol, wobei oft Hämolyse zu bemerken ist, lecithinspaltende Enzyme vorhanden sind, ist nicht untersucht. Es ist nicht gerade unwahrscheinlich, daß die Fermente, die Lecithin spalten, mit denen für phosphorfreie Glyceride nicht identisch sind. Ich fand, daß bei Darstellung ohne Alkohol das Präparat stärker fettspaltend wirkt als bei Anwendung von Alkohol. In einer Verdünnung, wo kein fettspaltendes Agens mehr nachweisbar ist, wirkt die ohne Alkohol dargestellte Lipase noch fettspaltend. Die anfangs neutrale Reaktion des Gemisches wird während der Einwirkung allmählich sauer. Der Grad der Acidität ist in der Regel nicht sehr stark, Dies kann jedoch leicht durch die Annahme erklärt werden, daß mit der Zeit die in Freiheit gesetzte Säure zum Teil durch die Alkalisalze neutralisiert wird, welche der Lipase von der Darstellung her anhaften oder die mit dem Serum eingeführt werden. Es wäre ein vollkommener Fehlschuß, allein infolge des geringen Grades der Acidität anzunehmen, daß die Hämolyse nicht durch die abgespaltene Fettsäuren verursacht wären. Wegen der gleichzeitigen Bildung von löslichen Seifen der Ölsäure tritt eine Verminderung der Acidität, aber eine Zunahme in Stärke und Schnelligkeit der Reaktion bis zur kompletten Hämolyse ein. An dieser Stelle sei bemerkt, daß Kyes' letzte Methode¹⁾ zur möglichst ergiebigen Darstellung von Kobralecithid in einer Entfernung der durch das

¹⁾ Kyes, Über die Lecithide des Schlangengiftes. Diese Zeitschr. 4. 101, 1907.

Gift aus dem Lecithinmolekül abgespaltenen Ölsäure und deren Neutralisation mit Natronlauge beruht. Nach wiederholter Schüttelung und Neutralisation wird der in Chloroform lösliche Anteil mit einer genau gemessenen Menge Salzsäure versetzt,¹ wobei das Natrium der Ölsäure entzogen wird.

Diese letzte Vorsichtsmaßregel scheint besonders wichtig zu sein, insofern als Lecithid und Natriumoleat beide stark hämolytisch wirken und dieselben Löslichkeitsverhältnisse haben, zumal im unreinen Zustande.

Das Fettspaltungsvermögen des Pankreaspräparates verschwindet beim Erwärmen auf 60° C. Bei 56° findet bereits beträchtliche Schwächung statt. Andererseits kann Lipase im trocknen Zustand ohne Einbuße an ihrer Kraft auf 110° C erhitzt werden. Der Hämolyse, die durch Lipase und Fette hervorgerufen wird, fehlt die Spezifität.

Blutserum als Komplement für die lipolytische Hämolyse.

Das Serum von Hund und Meerschweinchen ist fähig, bei Gegenwart von Pankreaslipase Hämolyse zu bewirken. Serum vom Rind ist dazu weniger imstande, wahrscheinlich infolge der Gegenwart einer die freien Fettsäuren schnell neutralisierenden Substanz oder infolge der Armut an Oleinfetten. In den dazu geeigneten Fällen liefert das Serum Fette für die Hämolyse, und diese werden dabei gleichzeitig verseift.

Hemmung der lipolytischen Hämolyse durch bestimmte Salze.

Cyankalium und Fluornatrium heben in einer Verdünnung von 1 : 10 000 den Eintritt der Hämolyse auf. Das einmal gebildete hämolytische Agens ist kochbeständig und gegen Neutralisation mit Alkalihydroxyd oder Carbonat widerstandsfähig. Die Hydroxyde und Carbonate der Erdalkalien hemmen stark.

Beschleunigung der hämolytischen Lipolyse durch bestimmte Salze.

Bekannt ist die beschleunigende Wirkung der gallensauren Salze auf die Fettspaltung¹⁾ u. ²⁾. Es lag nahe, den Einfluß

¹⁾ Magnus, Die Wirkung synthetischer Gallensäuren auf die pankreatische Fettspaltung. Zeitschr. f. physiol. Chem. 48, 376, 1906.

²⁾ Loevenhart und Souder, On the effect of bile upon hydrolysis of esters by pancreatic juice. Journ. of biol. Chem. 2, 415, 1907.

dieser Salze auf die lipolytische Form der Hämolyse zu prüfen. Es ist festgestellt, daß taurocholsaures und glykocholsaures sowie cholsaures Natrium stark hämolytisch sind; wenn man sie jedoch in $\frac{1}{500}$ Normallösung anwendet, sind sie an sich nicht hämolytisch, aber imstande, den Eintritt der Hämolyse zu fördern. Mangansulfat gab weniger günstige Resultate.

Die lipolytische Form der Hämolyse ist, obgleich die chemische Seite der Frage einfach ist, nicht ohne Interesse im Hinblick auf die komplexen biologischen Hämolysine. Der vorliegende Fall ist vielleicht das erste Beispiel, in dem ein gut bekanntes, wenn nicht gut definiertes Ferment, wie die Lipase, in ursächlichen Zusammenhang mit den Erscheinungen der Hämolyse gebracht ist. Besondere Aufmerksamkeit verdient die Tatsache, daß eine auffallende Ähnlichkeit zwischen der lipolytischen Hämolyse und der gewöhnlichen durch „Amboceptoren-Komplemente“ besteht, da in beiden Fällen zwei verschiedene Komponenten zusammenwirken. Lipase und Serumamboceptor sind beide, wenn auch in verschiedenem Grade, thermolabil. Beide geben zur immunisatorischen Bildung von Antikörpern Anlaß. In beiden Fällen unterstützen bestimmte Serumbestandteile ihre Wirkung auf die Blutkörperchen, obgleich die Komplementsubstanzen im einzelnen Falle sehr verschieden sein mögen. Es ist nicht unwahrscheinlich, daß das jüngst von Friedemann¹⁾ beschriebene komplexe Hämolysin aus Pankreassaft und Drüse teilweise mit der Pankreaslipase identisch ist. Ein anderer interessanter Gesichtspunkt ist der, daß zwei an sich unwirksame Substanzen durch Mischung aktiv werden, sowie der, daß die wichtigste Körperflüssigkeit, das Blut, eine hinreichende Menge Fett enthält, um nach Einwirkung von Pankreaslipase Blutkörperchen zu zerstören. Sollte Lipase durch Zufall oder unter pathologischen Verhältnissen in die Blutbahn gelangen, so besteht die Gefahr der lipolytischen Hämolyse; die Blutlipase, welche höhere Glyceride nicht spaltet, kommt hierfür nicht in Betracht.

1) Friedemann, Über ein komplexes Hämolysin der Bauchspeicheldrüse. Deutsche med. Wochenschr. 33, 585, 1907.

Über gewisse chemische Komplementsubstanzen.

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Die Frage der natürlichen und erworbenen Immunität eines vielzelligen Organismus gegen fremde Zellelemente, besonders gegen Bakterien und Blutkörperchen, hat während der letzten 20 Jahre den Gegenstand sehr sorgfältiger Untersuchungen gebildet. Während viele wichtige Tatsachen und außerordentlich geistreiche Theorien über die Art der defensiven Anpassung und über die Natur der Schutzkörper veröffentlicht worden sind, ist nichts Entscheidendes über die Chemie der sogenannten Immunitätsreaktionen bekannt geworden. Bevor ich die Resultate meiner eigenen Experimente und die darauf sich aufbauende Ansicht über die Natur von Alexin oder Komplement äußere, möchte ich eine kurze Übersicht über gewisse grundlegende Tatsachen und einige Theorien, die diesen Gegenstand beherrscht haben und noch beherrschen, geben.

Metschnikoffs Ansicht, daß Phagocytose das hauptsächlichste Schutzmittel des Organismus gegen das Eindringen von Bakterien sei, erhielt eine starke Erschütterung durch die bemerkenswerte Entdeckung der bakterientötenden Eigenschaft des zellenfreien Blutserums durch Nuttall.¹⁾ Dieser Befund wurde bald von Nissen²⁾,

¹⁾ Nuttall, Experimente über die bakterienfeindlichen Einflüsse des tierischen Körpers. Zeitschr. f. Hygiene 4, 353, 1888.

²⁾ Nissen, Zur Kenntnis der Bakterien vernichtenden Eigenschaft des Blutes. Zeitschr. f. Hygiene 4, 487, 1889.

Behring¹⁾, Behring und Nissen²⁾, Fodor³⁾, Buchner⁴⁻⁵⁾ und anderen bestätigt.

Der Grund der bactericiden Fähigkeit wurde von Buchner⁶⁾ einem aktiven (lebenden) Eiweiß zugeschrieben, wobei gewisse alkalische Salze eine wichtige Rolle spielten. Hankin⁷⁾ meinte, daß sie von dem Vorhandensein gewisser, besonders aktiver Proteine im Serum herrühre. Fodor⁸⁾, Behring⁹⁾, Nissen¹⁰⁾, Löwit¹¹⁾ und Pane¹²⁾ schrieben die antibakterielle Eigenschaft der Alkalinität des Blutes zu. Das Verschwinden dieser Eigenschaft bei Hitze von 56° C führte zur Aufstellung der Kohlensäuretheorie von Christmas¹³⁾; Hamburger¹⁴⁾ war ähnlicher Meinung und glaubte, daß CO₂ eine diffusible Alkaliverbindung aus Alkali-Albuminat bilde, dementsprechend die bactericide Fähigkeit des Venenblutes stärker als die des Arterienblutes war.

1) Behring. Über die Ursache der Immunität von Ratten gegen Milzbrand. *Centralbl. f. klin. Med.* 9, 681, 1888.

2) Behring und Nissen, Über bakterienfeindliche Eigenschaft verschiedener Blutarten. *Zeitschr. f. Hygiene* 8, 412, 1890.

3) Fodor, Die Fähigkeit des Blutes, Bakterien zu vernichten. *Deutsche med. Wochenschr.* 13, 745, 1887.

4) Buchner, Über die bakterientötende Wirkung des zellfreien Blutserums. *Centralbl. f. Bakt.* 5, 817, 1889.

5) Derselbe, Über die nähere Natur der bakterientötenden Substanz im Blutserum. *Centralbl. f. Bakt.* 6, 561, 1889.

6) Derselbe, Zur Lehre von natürlicher Immunität. *Münch. med. Wochenschr.* 29, 1419, 1899.

7) Hankin, Über die schützenden Eiweißkörper der Ratte. *Centralbl. f. Bakt.* 9, 336, 1891.

8) Fodor, Neuere Untersuchungen über die bakterientötende Wirkung des Blutes und ihrer Immunisation. *Centralbl. f. Bakt.* 7, 753, 1890.

9) Behring, Beiträge zur Ätiologie des Milzbrandes. *Zeitschr. f. Hygiene* 6, 117, 1889.

10) Nissen, Zur Kenntnis der Bakterien vernichtenden Eigenschaft des Blutes. *Zeitschr. f. Hygiene* 6, 487, 1889.

11) Löwit, Über die Beziehungen der Leukocyten zur bactericiden Wirkung und zur alkalischen Reaktion des Blutes und der Lymph. *Ziegler's Beiträge* 22, 172, 1897.

12) Pane, Ricerche sulla sostanze battericide del siero di sangue. *Revista clinica e terapeutica* 14, 705, 1892.

13) de Christmas, Etude sur les substances microbicides. *Annales de l'Inst. Pasteur* 5, 487, 1891.

14) Hamburger, Über den heilsamen Einfluß von venöser Stauung und Entzündung im Kampfe des Organismus gegen Mikroben. *Centralbl. f. Bakt.* 20, 403, 1896.

Die Experimente von Emmerich und Tsuboi¹⁾ stehen vollständig im Widerspruch zu obiger Theorie. Diese Forscher fügten ein Quantum Natronhydrat hinzu, genügend, um die ganze Kohlensäure des Hundeserums zu binden, und dialysierten dann bis zum vollständigen Verschwinden des freien Alkalis. Die bactericide Kraft des dialysierten Serums war größer als die des ursprünglichen. Die Erhitzung auf 55° brachte keine Inaktivierung hervor. Dieses Phänomen wurde folgendermaßen erklärt: CO₂ setzt die Aktivität des bactericiden Proteins herab, da es die aktiveren Alkalialbuminate spaltet und sie fast inaktiv macht. Die Inaktivierung des frischen aktiven Serums bei 55° C ist dem Freiwerden von CO₂ aus Bicarbonaten und einer nachfolgenden Spaltung der Albuminate zuzuschreiben. Somit konnte in den oben angeführten Experimenten keine Inaktivierung stattfinden, da in dem Serum nach Hinzufügen von NaOH weder Bicarbonate noch Kohlensäure vorhanden waren. A. Fischer²⁾ suchte die Ursache der Bakterienvernichtung in rein physikalischen Bedingungen, wie in osmotischer Zerstörung, während Baumgarten³⁾ sie für ein Hungerphänomen verbunden mit Fischers plasmolytischen Veränderungen hielt. Finckh⁴⁾ fand, daß das Hinzufügen von Nährsubstanzen, wie Pepton und Zucker, und gewisser Magnesiumsalze zum Serum die bactericide Kraft beseitigte. Obgleich solche Versuche zur gleichen Zeit von Baumgarten zur Stütze seiner Ansicht angestellt wurden, so führten doch spätere Untersuchungen zu einer vollständig anderen Erklärung. Man wies nach, daß Pepton antikomplementär wirkt, sowohl in vivo als in vitro, während Magnesiumsalze die Wirkung der Komplemente sehr stark beeinträchtigen. Nolf⁵⁾ wider-

1) Emmerich und Tsuboi, Über die Erhöhung und Regenerierung der mikrobiciden Wirkung des Blutserums. *Centralbl. für Bakt.* **13**, 575, 1893.

2) A. Fischer, Die Empfindlichkeit der Bakterienzellen und das bactericide Serum. *Zeitschr. f. Hygiene* **35**, 1, 1900.

3) Baumgarten, Zur Lehre von den natürlichen Schutzmitteln des Organismus gegenüber Infektion. *Berl. klin. Wochenschr.* **37**, 136, 162, 192, 1900.

4) Finckh, Aufhebung der sog. bactericiden Wirkung des Blutserums durch Zusatz von Nährstoffen. *Centralbl. f. Bakt.* **28**, 694, 1900.

5) Nolf, Le mécanisme de la globulyse. *Annales de l'Inst. Pasteur* **14**, 656, 1900.

sprach Buchners Ansicht von der proteolytischen Fermentnatur des Alexins und hielt es für ein einfaches hydratisierendes Agens und nicht für ein Enzym. Gleichzeitig bemühten sich Metschnikoff und seine Schüler¹⁻³⁾ Beweise dafür zu finden, daß Alexin ein Produkt zerfallener Leukocyten sei und daher im zirkulierenden Blutplasma nicht vorkomme. Buchner glaubte hingegen, daß Alexin ein physiologisches Produkt der Leukocyten und ein normaler Bestandteil des Plasmas wäre. Lambotte, Falloise³⁾, Lambotte und Stiénon⁴⁾ befürworteten die Präexistenz des Alexins im Blute, und diese Ansicht wurde durch die Experimente von Gruber und Futaki⁵⁾ unterstützt. Die letzten Forscher schreiben — während sie die Absonderung bacterioider Substanzen seitens gewisser Leukocyten zugeben — den Ursprung der bactericiden Kraft des klaren Blutserums den Blutkörperchen zu.

Man hat lange behauptet, daß Leukocyten die Quelle von Alexin wären. Diese Ansicht geht auf Metschnikoff's Phagocytoselehre zurück und wurde von vielen Forschern, besonders von Buchner, Schattenfroh und Bail, angenommen. Schattenfroh⁶⁻⁷⁾ erzielte einen ziemlich starken bactericiden wässerigen Auszug aus den Leukocyten eines Aleuronatexsudats durch Frierenlassen und Wiederauftauen. Das Extrakt zeigte eine größere Hitzebeständigkeit⁸⁾ als Alexin. Mononucleäre Zellen

1) Gongou, Contribution à l'étude de l'origine de l'alexine des serums normaux. Annales de l'Inst. Pasteur 15, 68, 232, 1901.

2) Relms, Sur le mode d'action des cytolysines in vivo. Compt. rend. de la Soc. de Biol. 56, 609, 1904.

3) Falloise, Sur l'existence de l'alexine hémolytique dans le plasma sanguin. Bull. Acad. roy. Belgique 1903, 521.

4) Lambotte und Stiénon, Alexine et Leucocytes. Centralbl. f. Bakt. 40, 224, 393, 503, 1905.

5) Gruber und Futaki, Über die Resistenz und über die Herkunft der milzbrandfeindlichen Stoffe. Münch. med. Wochenschr. 54, 251, 1907.

6) Schaffenfroh, Über das Vorhandensein von bacterioiden Stoffen in Leukocyten und deren Extraktion. Münch. med. Wochenschr. 44, 4, 114, 1897.

7) Derselbe, Arch. f. Hygiene 31, 1, 1897.

8) Derselbe, Neuere Erfahrungen über den bakterienfeindlichen Stoff der Leukocyten. Münch. med. Wochenschr. 45, 353, 1898.

enthielten die zerstörende Substanz nicht¹⁾. Bail bestätigte in einer Reihe von Experimenten²⁻⁴⁾ Schattenfrohs Entdeckung. Schattenfroh⁵⁾ wies nach, daß ein Leukocytenextrakt bactericid sein kann, ohne hämolytisch zu sein. Beide Autoren fanden Nucleohiston nicht bakteriolysisch. Außer dieser Tatsache, daß der Leukocytenauszug bactericide Substanzen enthält, ist nichts über die chemische Natur dieser Körper und ihre Beziehung zu bactericidem Serum bekannt. Pirenne⁶⁾ unterschied zwei bactericide Substanzen im Rattenserum. Die eine ist als bei 0° C aktiv beschrieben und ist in höherem Grade wärmebeständig. Die andere ist bei 0° C inaktiv und erzeugt in einem mit diesem Serum behandelten Organismus einen Antikörper. Ihre Thermolabilität ist dieselbe wie die von Alexin im allgemeinen.

Eine neue Förderung erfuhr das Studium dieses Gebietes durch Pfeiffers Entdeckung der zusammengesetzten (dualen) Natur des Immunerums. Er fand zuerst⁷⁻¹⁰⁾, daß das Immuneserum, wenn es einmal seine Aktivität, sei es durch Erhitzung auf 55° C oder durch langes Stehen verloren hat, wieder aktiv wird, wenn es in den Tierkörper eingeführt wird; ein inaktives normales Ziegenserum kann auf diese Weise reaktiviert werden. Diese Entdeckungen bewirkten, daß von Buchners Alexin zwei

1) Schattenfroh, Über hitzebeständige bactericide Leukocytenstoffe. Münch. med. Wochenschr. 45, 1109, 1898.

2) Bail, Über das Freiwerden der bactericiden Leukocytenstoffe. Berl. klin. Wochenschr. 34, 887, 1897.

3) Derselbe, Schutzstoffe gegen Staphylokokkeninfektion. Berl. klin. Wochenschr. 35, 921, 1898.

4) Derselbe, Über Inaktiviertheit leukocytenreicher Exsudate. Berl. klin. Wochenschr. 35, 481, 1898.

5) Schattenfroh, Weitere Untersuchungen über die bakterienfeindlichen Stoffe der Leukocyten. Arch. f. Hygiene 35, 133, 1899.

6) Pirenne, Sur les alexines et les substances microbicides du serum normal. Centralbl. f. Bakt. 36, 256, 1904.

7) Pfeiffer und Wassermann, Untersuchungen über das Wesen der Choleraimmunität. Zeitschr. f. Hygiene 14, 46, 1893.

8) Pfeiffer und Isaëff, Über die spezifische Bedeutung der Choleraimmunität. Zeitschr. f. Hygiene 17, 355, 1894.

9) Pfeiffer, Weitere Untersuchungen über das Wesen der Choleraimmunität und über spezifische bactericide Prozesse. Zeitschr. f. Hygiene 18, 1, 1894.

10) Derselbe, Differentialdiagnose der Vibrionen der Cholera asiatica mit Hilfe der Immunisierung. Zeitschr. f. Hygiene 19, 85, 1895.

deutlich verschiedene Bestandteile unterschieden wurden. Metschnikoff¹⁾ und Bordet²⁾ beobachteten den Eintritt desselben Phänomens *in vitro*. Bordet³⁻⁵⁾ klärte beim Arbeiten mit hämolytischem Serum den Mechanismus der Zellauflösung auf und fand, daß bei der Hämolyse zwei Prinzipien in Betracht kommen. Das eine ist ein Immunisierungsprodukt und besitzt größere Wärmebeständigkeit; seine Wirkung besteht darin, die Blutkörperchen spezifisch zu sensibilisieren und für die auflösende Wirkung des normal im Serum vorhandenen Alexins empfänglich zu machen. Das andere — von Bordet Alexin benannt — übt seine lösende Wirkung auf die Blutkörper aus, wenn letztere spezifisch sensibilisiert sind, aber nicht auf die nicht behandelten Zellen und verschwindet bei 55° C oder spontan, wenn es einige Tage bei Zimmertemperatur steht. Bordet wies nach, daß ein durch Erwärmung auf 55° C oder durch Alter inaktiviertes Serum wieder aktiv gemacht werden kann, wenn man im normalen Serum vorhandenes Alexin hinzufügt. Diese wichtige Entdeckung wurde von Ehrlich und Morgenroth⁶⁾ bestätigt und weiter ausgeführt, die ihre Resultate fast gleichzeitig veröffentlichten. Sie gaben an, daß die Art der Wirkung des aktiven Serums genau die gleiche wie die des Immunserums wäre und von zwei Komponenten abhinge. Bordet gebraucht die Bezeichnung „*substance sensibilisatrice*“ für den sensibilisierenden Bestandteil, während Ehrlich und Morgenroth die Ausdrücke Zwischenkörper oder Amboceptor anwenden. Buchners Alexin ist nicht mehr identisch mit dem Alexin von Bordet; letzteres ist dasselbe, wie das Komplement von Ehrlich und Morgenroth.

1) Pfeiffer, Weitere Mitteilungen über die spezifischen Immunkörper der Cholera. Zeitschr. f. Hygiene 20, 198, 1895.

2) Metschnikoff, Recherches sur la destruction extracellulaire des bactéries. Annales de l'Inst. Pasteur 9, 433, 1895.

3) Bordet, Sur le mode de l'action des serums preventifs. Annales de l'Inst. Pasteur 10, 193, 1896.

4) Derselbe, Sur l'agglutination et la dissolution des globules rouges par le serum d'animaux injectes de sang defibriné. Annales de l'Inst. Pasteur 12, 688, 1898.

5) Derselbe, Agglutination et dissolution des globules rouges par le serum. Annales de l'Inst. Pasteur 13, 273, 1899.

6) Ehrlich und Morgenroth, Zu Ehrlichs Immunitätsforschung. Berlin 1904.

M. Wilde¹⁾ und von Dungern²⁾ fanden, daß Alexin oder Komplement von verschiedenen Bakterien, Hefen und vielen nicht-spezifischen Zellen absorbiert und verbraucht wird. Levene und Baldwin³⁾ fanden, daß Komplement durch Nucleinsäure, Tuberkulinsäure, sowie durch Glykogen und andere Zellen- und Gewebsbestandteile inaktiviert wird. Landsteiner und Stankowic⁴⁾ fanden, daß Komplement von Casein, Kaolin, Cholesterin und von anderen organischen und anorganischen Substanzen absorbiert wird. Die Absorption des Alexins durch spezifische Präcipitate oder sensibilisierte Zellen wurde zuerst von Bordet⁵⁾ beobachtet und dann von Gengou⁶⁾, Gay⁷⁾ und Moreschi⁸⁾ bestätigt. Dieses Phänomen wurde für diagnostische Zwecke in der forensischen Medizin angewandt (Neisser-Sachs'sche Komplementablenkungsprobe⁹⁾ und zum Nachweis von spezifischen Antikörpern oder Antigenen¹⁰⁾.

Tarasévitch¹¹⁾ versuchte die Herkunft des Alexins zu bestimmen, das nach Metschnikoffs Theorie in den Leukocyten vorhanden ist und seine Wirkung nach dem Zerfall der Zellen offen-

1) Wilde, Über die Absorption der Alexine durch abgetötete Bakterien. Berl. klin. Wochenschr. 36, 481, 1899.

2) v. Dungern, Beiträge zur Immunitätslehre. Münch. med. Wochenschr. 47, 677, 1900.

3) Levene und Baldwin, On the anticomplementary action of some cell and tissue constituents. Journ. of Med. Res. 12, 205, 1904.

4) Landsteiner und Stankowic, Über die Bindung von Komplementen durch suspendierte und kolloid gelöste Substanzen. Centralbl. f. Bakt. 47, 353, 1906.

5) Bordet, Les serums hémolytiques et leur antitoxines. Annales de l'Inst. Pasteur 14, 257, 1900.

6) Gengou, Sur les sensibilisatrices des serums actifs contre les substances albuminoïdes. Annales de l'Inst. Pasteur 16, 734, 1902.

7) Gay, The fixation of alexins by specific precipitates. Centralbl. f. Bakt. 39, 603, 1905.

8) Moreschi, Zur Lehre von den Antikomplementen. Berl. klin. Wochenschr. 42, 1181, 1905.

9) Neisser und Sachs, Ein Verfahren zum forensischen Nachweise der Herkunft des Blutes. Berl. klin. Wochenschr. 42, 1388, 1905.

10) Wassermann und Bruck, Exper. Studien über die Wirkung von Tuberkelbacillenpräparaten auf den tuberkulös erkrankten Organismus. Deutsche med. Wochenschr. 32, 449, 1906.

11) Tarasévitch, Sur les cytases. Annales de l'Inst. Pasteur 21, 127, 1902.

bart, und fand, daß polynucleäre Leucocyten, welche Mikroben aufnehmen, sogenannte Mikrophagen, ein Alexin abgeben, das besonders Bakterien vernichtet, während mononucleäre Leucocyten, die meistens nichtbakterielle Zellelemente enthalten, ein gegen die Blutkörper wirkendes Alexin ergeben. Das erstere ist Mikrocytase benannt und das letztere wegen seiner Abstammung aus den Makrophagen, Makrocytase. Die Cytasen sind thermolabil, jedoch etwas widerstandsfähiger als Serumcytase oder Alexin. Korschun und Morgenroth¹⁾ erklärten in ganz anderer Weise die Wirkung der Extraktlysine verschiedener Eingeweide- und drüsigen Organe. Im Gegensatz zu Tarasévitch fanden diese Autoren, daß die Extrakte verschiedener Organe größere oder kleinere Mengen hämolytischer Substanzen enthalten, denen Spezifität fehlt und Thermostabilität zukommt.

Die hämolytischen Grundstoffe sind in Alkohol und Wasser löslich. Sie sind bei 0° C aktiv und ergeben keinen Antikörper bei der Immunisierung. Woelfel²⁾ erhielt aus Blutserum einen hämolytischen Anteil durch Alkoholextraktion. Diese Fraktion war nicht spezifisch, widerstand der Hitze und war bei 0° C aktiv. Die chemische Natur dieses hämolytischen Anteils ist nicht erforscht worden.

Ogata³⁾ gab an, daß man einen antibakteriellen Niederschlag aus Hundeserum durch Alkohol und Äther fällen könne. Es ist nach seinen Angaben sehr thermolabil, leicht durch schwache Säure und Alkali zu inaktivieren und durch Verdauungsfermente zerstörbar. Petermann⁴⁾ vermochte Ogatas Angaben nicht zu bestätigen. De Christmas⁵⁾ fand auch, daß eine Alkoholfällung aus Serum auf Bakterien wirkt.

1) Korschun und Morgenroth, Über die hämolytische Eigenschaft von Organextrakten. Berl. klin. Wochenschr. 39, 870, 1902.

2) Woelfel, Alcohol soluble haemolysins in blood serum. Journ. of Infect. Diseases 2, 97, 1905.

3) Ogata, Über die bakterienfeindliche Substanz des Blutes. Centralbl. f. Bakt. 9, 597, 1891.

4) Petermann, Sur la substance bactericide du sang. Annales de l'Inst. Pasteur 5, 1506, 1891.

5) de Christmas, Étude sur les substances microbiocides. Annales de l'Inst. Pasteur 5, 487, 1891

Conradi¹⁾ isolierte aus autolysierten Geweben mittels Alkohol und Äther eine stark bactericide Fraktion. Das Extrakt war wärmebeständig, in Alkohol löslich, in Äther unlöslich, in kaltem Wasser mit leichter Opalescenz löslich, die beim Kochen verschwand und beim Abkühlen wiederkehrte. Es wird als dialysierbar geschildert und als durch Chamberlandkerzen filtrierbar sowie beim Stehen langsam zerstörbar.

A. Kossel²⁾ stellte fest, daß Nucleinsäure bactericid ist, während H. Kossel³⁾ Protamin und sein Carbonat fähig fand, Bakterien zu töten. In Anbetracht der bactericiden Eigenschaft der Nucleinsäure sind die Beobachtungen von Emmerich, Loew und Korschun⁴⁾ über die bactericide Wirkung der Nucleasen von besonderem Interesse. Die Tatsache, daß Nucleasen Nuclein spalten, — wobei Nucleinsäure frei wird und bactericide Eigenschaften auftreten, hat auffallende Ähnlichkeit mit einer lipolytischen Form der Hämolyse, die kürzlich von Noguchi⁵⁾ beschrieben wurde; er beobachtete, daß Pankreassaft oder -Gewebe, die sonst inaktiv bleiben, hämolytisch werden, wenn gewisse Mengen neutraler Fette in der Mischung vorhanden sind. Die Hämolyse wurde hier durch die Fettsäure hervorgebracht⁶⁾, die aus den Fetten durch Lipase abgespalten ist.

1) Conradi, Über die Bildung bacterizider Stoffe bei der Autolyse. Beiträge z. chem. Physiol. u. Pathol. 1, 193, 1901.

2) A. Kossel, Über die Lymphzellen. Deutsche med. Wochenschr. 20, 146, 1894.

3) H. Kossel, Über bacterizide Bestandteile tierischer Zellen. Zeitschr. f. Hygiene 27, 36, 1898.

4) Emmerich, Loew, Korschun, Die bakteriolytische Wirkung der Nucleasen und Nucleosen. Centralbl. f. Bakt. 31, 1, 1902.

5) Noguchi, A lipolytic form of haemolysis. Proc. of the Soc. for Exp. Biol. and Med. 1907; ferner diese Zeitschr. 6, 185, 1907.

6) Neuberg und Rosenberg (Berl. klin. Wochenschr. 44, 54, 1907) lenkten die Aufmerksamkeit auf eine mögliche Verwandtschaft zwischen Lipolyse, Hämolyse und Agglutination bei den von Schlangengiften und gewissen Pflanzentoxalbuminen bewirkten Erscheinungen. Später fanden Neuberg und Reicher (diese Zeitschr. 4, 281, 1907) eine größere lipolytische Wirkung bei gewissen Immunseris als bei normalen Seris derselben Arten. — Friedemann (Deutsche med. Wochenschr. 33, 585, 1907) beschreibt — allerdings ohne Andeutungen über eine Rolle der Lipase — eine komplexe Hämolyse durch Pankreassaft oder -Gewebe. — Wohlgemuth (diese Zeitschr. 4, 271, 1907) gab an, daß gewisse Bestandteile des Pankreas sich mit Lecithin zu einem Lecithid verbinden.

Buchner¹⁻³⁾ fand, daß durch Hinzufügen einer großen Menge destillierten Wassers oder durch Dialysieren gegen fließendes Wasser die bactericide Eigenschaft des Serums verschwand und daß sie sich nach Hinzugabe von Ammoniaksalzen besser als im unbehandelten Serum erhielt. **Shibayama**³⁾ reaktivierte das normale Serum, das für fremde Blutkörper durch Dialyse unmerklich geworden war, durch Zusatz kleiner Mengen Natriumcarbonat. Die hämolytische Kraft des Immunserums ist durch die Dialyse unbeeinflusst gefunden. **Ferrata**⁴⁾ gab an, daß Komplement durch Dialyse gegen Wasser in zwei inaktive Komponenten zerlegt werden kann. Diese beiden Komponenten werden wieder wirksam, wenn man sie miteinander in Salzlösung vermischt. Andererseits fanden **Sachs und Teruuchi**⁵⁾, daß die hämolytische Wirkung des normalen Meerschweinchenserums in salzfreiem Medium stärker als in salzhaltigem ist. Aber das Komplexsystem, das aus einem Immunamboceptor und einem passenden Komplement bestand, ergab ein ganz entgegengesetztes Resultat, nach welchem das Komplement in einem salzfreien Medium eine irreversible Inaktivierung erfuhr. Diese Inaktivierung, oder richtiger gesagt, Zerstörung des Komplements, schreiben sie der Wirkung eines fermentähnlichen Bestandteiles in dem angewandten Serum zu.

Das Vorhandensein hämolytischer und bakteriolytischer Komplemente in der Lymphe wurde von **Meltzer und Norris**⁶⁾ sowie von **Landsteiner**⁷⁾ nachgewiesen. Einengen zur Trockne bei niedriger Temperatur (20° C) nimmt dem Serum seine

¹⁾ Buchner, Über die Schutzstoffe des Serums. Berl. klin. Wochenschr. 29, 449, 1892.

²⁾ Derselbe. Die keimtötende, globulicide, und die antitoxische Wirkung des Blutserums. Munch. med. Wochenschr. 39, 119, 1892.

³⁾ Shibayama, Einige Experimente über Hämolyse. Centralbl. f. Bakt. 30, 760, 1901.

⁴⁾ Ferrata, Die Unwirksamkeit der komplexen Hämolyse in salzfreien Lösungen und ihre Ursache. Berl. klin. Wochenschr. 44, 366, 1907.

⁵⁾ Sachs und Teruuchi, Die Inaktivierung der Komplemente im salzfreien Medium. Berl. klin. Wochenschr. 44, 467, 250, 602, 1907.

⁶⁾ Meltzer und Norris, The bactericidal action of lymph taken from thoracic duct of the dog. Journ. of Exp. Med. 2, 701, 1897.

⁷⁾ Landsteiner, Antifermentative, lytische und antilutinierende Wirkungen des Blutserums. Centralbl. f. Bakt. 37, 290, 1900.

komplementäre Kraft nicht, und das Komplement widersteht in getrocknetem Zustand der Einwirkung von Temperaturen unter 135°C ¹⁾. Bei Bestimmung der Charakteristik der Komplemente ist der Einfluß verschiedener Säuren, Alkalien und Salze von großer Bedeutung²⁻⁴⁾. Der Einfluß von „Schutzwirkungen“ liefert, wie von Noguchi⁵⁾ beschrieben wurde, noch ein anderes Unterscheidungsmerkmal für das Komplement. Pfeiffer⁶⁾ fand, daß das Komplement zerstört wird, wenn man das Serum dem Sonnenlicht bei Vorhandensein von fluorescierenden Anilinfarben aussetzt.

Überblickt man die Literatur über Alexine und Komplemente, so findet man, daß sie durch folgende Eigenschaften charakterisiert sind:

1. Spontanes Verschwinden mit der Zeit;
2. Inaktivierung bei halbstündiger Erhitzung auf 55°C ;
3. Unwirksamkeit bei 0°C , trotz Vorhandenseins von Amboceptor (oder sensibilisierender Substanz);
4. Inaktivität bei Fehlen eines spezifischen Amboceptors, aber Wirksamkeit bei Vorhandensein desselben oder nach voraufgegangener geeigneter Sensibilisierung der Zellen;
5. Absorption durch nichtspezifische Zellelemente, durch sensibilisierende Zellen und Proteide sowie durch einige suspendierte und kolloidale Substanzen;
6. Hemmung durch gewisse normale Sera, sowohl frische als auf $55-60^{\circ}\text{C}$ erhitzte, und durch „Schutzwirkung“;
7. Empfindlichkeit gegen verschiedene Säuren, Alkalien und Salze;

1) Noguchi, On the influence of the reaction and of dessication upon opsonins. *Journ. of Exp. Med.* 9, 455, 1907.

2) Hektoen, The action of certain ions upon the lysin in human serum. *Trans. of the Chi. Path. Soc.* 10, 303, 309, 1903.

3) Manwaring, The action of certain salts on the complement in immune serum. *Journ. of Infect. Diseases.* 1, 112, 1904.

4) Noguchi, On the chemical inactivation and regeneration of complement. Read before the ann. Meeting of the Soc. of American Bacteriologists, 1906, December 28.

5) Derselbe, The thermostabile anticomplementary constituents of the blood. *Journ. of Exp. Med.* 8, 726, 1906.

6) Pfeiffer, Über die Wirkung des Lichtes auf Eosinblutgemische. *Wiener klin. Wochenschr.* 18, 221, 328.

8. Resistenz gegen Austrocknung und trockene Wärme;
9. Inaktivierung durch photodynamische Anilinfarbstoffe.

Bei der Alkohol- oder Alkoholätherextraktion von Serum sowie von Organ- und Gewebselementen erhält man Substanzen, die entweder hämolytisch oder baktericid sind, indem die verschiedenen Organe oder drüsigen Gewebe sowie das Blutserum wechselnde Mengen dieser lytischen Substanzen ergeben. Letztere sind durch folgende Eigenschaften charakterisiert:

1. Geringfügige Verminderung ihrer Wirksamkeit mit der Zeit;
2. Aktivität selbst nach langem Kochen;
3. Lytische Wirkung, die von einer zweiten Substanz unabhängig ist;
4. Wirksamkeit bei 0° C;
5. Mangelnde Spezifität;
6. Löslichkeit in Alkohol und kaltem Wasser unter Trübung, die beim Kochen verschwindet und beim Abkühlen wiederkehrt;
7. Aufhebung der Wirkung durch kleine Mengen normalen Serums.

Wenn man den Charakter der Komplemente und der alkohol-löslichen Extraktlysine vergleicht, so findet man nicht eine einzige gemeinschaftliche Eigenschaft. Man darf hieraus jedoch nicht schließen, daß sie notwendigerweise verschiedene Körper sind, solange der Vergleich unter verschiedenen Bedingungen angestellt worden ist. Bevor irgend ein Vergleich der Substanzen der beiden Klassen vorgenommen wird, sollte man die Beobachtungsbedingungen einander so ähnlich wie nur irgend möglich machen. Im vorliegenden Falle ist es angängig, fast die gleichen Bedingungen für Extraktlysine herzustellen, indem man sie einfach mit gewissen Mengen von Normalserum vermischt. In der Tat fand sich bald, daß die Extraktlysine mehrere der charakteristischsten Eigenschaften eines Komplements annehmen und einige der ihnen im freien Zustand eigentümlichsten verlieren, wenn diese Bedingungen erfüllt werden. Von jenen erworbenen Eigenschaften sind Thermolabilität bei 55° C, der Verlust der unabhängigen Wirksamkeit, Inaktivität bei 0° C, die komplementäre Funktion und endlich das spontane Verschwinden die wichtigsten. Es muß an dieser Stelle mit Nachdruck hervorgehoben werden, daß die Komplementärwirkung nur erlangt wird, wenn die Menge des Serums gerade ausreichend ist, um die vor-

handene lytische Kraft zu verdecken, und daß der Fortschritt der Hämolyse viel langsamer erfolgt, als bei der Reaktivierung durch genuines Serumkomplement. Bisher sind alle Versuche, diese Verschiedenheiten zu beseitigen, erfolglos geblieben. In der vorliegenden Arbeit war der Hauptzweck, der verfolgt wurde, die Feststellung der Verwandtschaft zwischen Komplement und Extraktlysinen, wofür auch physikalische und chemische Arten der Identifizierung gesucht wurden. Die chemische Natur der Extraktlysine wurde ebenfalls erforscht. In den folgenden Kapiteln will ich meine Arbeit in zwei Abschnitte teilen, von denen der eine sich mit den hämolytischen, der andere mit den bactericiden Erscheinungen beschäftigen soll.

A. Hämolytische Versuche.

Die thermostabilen hämolytischen Elemente des Blutes und der Organe und ihre chemische Identifizierung.

Blut, Leber, Niere und Milz von Hund, Rind und Kaninchen wurden mit mehreren Volumen 95 prozentigen Alkohols eine Woche lang bei 45—50° C extrahiert. Vor der Extraktion wurden die Organe zerkleinert und mit physiologischer Kochsalzlösung in eine dicke Emulsion verwandelt. Der in Alkohol lösliche Teil wurde von dem Coagulum getrennt und bei 40° C abgedampft. Der Rückstand wurde nach Verdunstung des Alkohols mit warmem Alkohol von 70° C extrahiert und die Lösung wieder abgedampft. Die getrocknete Masse wurde dann mit Äther ausgezogen, der alle Fettsäuren, neutralen Fette, Cholesterin und seine Ester sowie phosphorhaltige Fette auszog. Der in Äther nicht lösliche Teil war in der Regel klebrig, leicht gelblich und unansehnlich. Er löste sich leicht in warmem Alkohol, langsam in kaltem Alkohol und Wasser. In kaltem Wasser zeigte er leichte Opalescenz, die schnell beim Kochen verschwand und beim Abkühlen wiederkehrte. Er löste sich auch in Chloroform mit schwacher Opalescenz. Die Reaktion einer Lösung dieser Fraktion in Wasser oder Salzlösung war gegen Lackmus neutral. Fällung mit Bleiacetat und nachfolgende Äther-Extraktion entfernten den größeren Teil dieser Fraktion. Der Extrakt ergab nach Vertreibung des Äthers und Behandlung mit Schwefelwasserstoff wechselnde Mengen von Fettsäuren,

besonders von Ölsäure. Jede starke Säure bringt in dieser Fraktion eine milchige Treibung hervor, die zweifellos auf dem Freiwerden von Fettsäuren beruht. Osmiumsäure schwärzt die Lösung allmählich, während Phosphorwolframsäure einen dicken weißlichen Niederschlag und Brom eine gelbliche Wolke hervorrief. Die Millons Probe war negativ. Calcium- und Bariumsalze riefen stärkere oder schwächere Niederschläge hervor. Chemisch stellte diese Fraktion ein Gemisch verschiedener Seifen dar. Bei biologischer Prüfung zeigte sie eine mächtige hämolytische Wirkung nicht spezifischer Natur. Die Erdalkalien machten sie inaktiv. Die Wirkung hörte bei 0° C nicht auf und widerstand dem Kochen, wurde aber leicht durch Hinzufügen einer adäquaten Menge indifferenten Serums aufgehoben. So ist es also klar, daß diese Fraktion mit den Extraktlysinen, die von gewissen Forschern bei vielen biologischen Reaktionen beschrieben wurden, identisch war. Die Menge des Alkoholextrakts und die hämolytischen Eigenschaften sind bei den Organen und Arten verschieden. Die größte Menge erhält man vom Hunde, die geringste vom Rind. Das Blut des Hundes ergab am meisten, die Leber am wenigsten; Niere und Milz ergaben ungefähr dieselbe Menge. So wies das Extraktlysin des Hundebutes, wenn es zum Originalvolumen in 0,9% NaCl-Lösung gelöst war, wobei der Gehalt etwa 0,1% betrug, eine solche hämolytische Kraft auf, daß 0,1 ccm der erhaltenen Lösung Hämolyse von 2,0 ccm einer fünfprozentigen Suspension gewaschener Hundebutkörper herbeiführten. Die Blutkörper von Rind und Kaninchen sind widerstandsfähiger als die von Meerschweinchen und Hunden. Die Extraktlösungen der Niere und Milz waren weniger konzentriert und waren in größeren Mengen erforderlich, um dieselbe Wirkung hervorzubringen. Die Leberextraktlösung war so schwach, daß 1 ccm nötig war, um denselben Wirkungsgrad hervorzubringen wie 0,1 ccm der Blutextraktlösung. Die Menge der alkohollöslichen Lysine war im Rinderblut viel geringer als im Blute von Hunden und Kaninchen. Bei der letzten Art war sie fast $2\frac{1}{2}$ mal geringer als beim Hundebut, war aber zweimal so groß wie beim Rinderblut.

Obgleich die definitive chemische Zusammensetzung dieser Fraktion nicht ins Detail festgestellt wurde, so besteht doch kein Zweifel darüber, daß sie verschiedene lösliche und schwer lös-

liche Seifen enthält, die aus dem Blute oder den Organen herrühren. Späteren Forschungen bleibt noch die Frage vorbehalten, ob in der Fraktion gewisse Seifenbestandteile vorhanden sind, deren Radikale komplizierter sind als die bisher bekannten entsprechenden anorganischen oder organischen Reste von Seifen. Mehrere Jahre sind vergangen, seit ich die Aufmerksamkeit auf die außergewöhnliche hämolytische Kraft des ölsauren Natrons lenkte¹⁾, und ich war daher nicht überrascht, eine starke hämolytische Kraft in der Fraktion konzentriert zu finden, die sich aus verschiedenen Seifen zusammengesetzt erwies. Um die Extraktlysine unter die Seifen bekannter Konstitution einzureihen, wurde eine systematische Untersuchung der hämolytischen Wirkung verschiedener anorganischer und organischer Seifen unternommen. Die Resultate zeigt die Tabelle I.

Tabelle I.

0,1proz. Seifenlösung und 2 cem 5proz. Aufschwemmung von Rinderblutkörperchen.

Menge der Seifen- lösung in cem	Ammonium- oleat	Neurin- oleat	Natrium- oleat	Magne- siumoleat	Calcium- oleat	Natrium- stearat	Magne- sium- stearat	Calcium- stearat
2								c. H.
1							c. H.	keine H.
0,7								
0,5					c. H.	c. H.		
0,4					Spur v. H.	keine H.		
0,3					keine H.			
0,2								
0,15								
0,1				c. H.				
0,07			c. H.	Spur v. H.				
0,05	c. H.	c. H.	Spur v. H.	keine H.				
0,04	starke H.	Spur v. H.	keine H.					
0,03	keine H.	keine H.						
0,02								
0,015								
0,01								

Aus den vorhergehenden Versuchen haben wir die Tatsache abgeleitet, daß die löslichen Seifen am aktivsten sind, während bei den fast unlöslichen Calciumseifen nur eine mehr

¹⁾ Noguchi, On certain thermostabile venom activators. Journ. of Exper. Med. 8, 1906.

oder weniger schwache Wirkung beobachtet wird. Die Oleate sind fast zehnmal stärker hämolytisch als die entsprechenden Stearinseifen. Nach dieser Tatsache zu urteilen, müßten die Extraktlysine hauptsächlich aus Oleaten und in viel geringerem Maße aus löslichen Stearaten bestehen.

Gleich den Extraktlysinen sind die Seifen coetostabil, alkohol-löslich, meist ätherunlöslich, in kaltem oder heißem Wasser unter leichtem Opalescieren löslich, nicht spezifisch, von unabhängiger Wirksamkeit, bei 0° C aktiv, nicht schnell zerlegbar; sie geben reichlich Niederschläge mit Phosphorwolframsäure und Brom und werden durch starke Säuren, die Fettsäuren freimachen, zersetzt oder gehen aus einem aktiven, löslichen in einen inaktiven, unlöslichen Zustand über, wenn man sie mit Calcium- oder Bariumsalzen vermischt. Eine kleine Menge Serum paralysiert leicht die hämolytische Wirkung der reinen Seifen.

Inaktivierung der Extraktlysine und Seifen durch Serum.

Die Tatsache, daß alle höheren Acrylsäuren eine viel größere hämolytische Kraft als irgend welche Mineral- oder organischen Säuren besitzen und daß ihre löslichen Seifen viel schnellere, wenn nicht stärkere, hämolytisch wirkende Fähigkeiten besitzen, ist nicht neu¹⁾. Jetzt erhebt sich natürlich die Frage, wie solche kräftig wirkenden hämolytischen Substanzen im Blute vorhanden sein können, ohne die ihnen innewohnende vernichtende Wirkung auf die Blutkörperchen auszuüben. Wie erwähnt, gaben Korschun und Morgenroth²⁾, welche die alkohollöslichen, coetostabilen Extraktlysine verschiedener Organe beschrieben, zu gleicher Zeit an, daß eine geringe Menge Serum die hämolytische Wirkung dieser Fraktionen aufhebt. Diese interessante Beobachtung ist von mir in der vorliegenden Arbeit völlig bestätigt worden. Weiter fand ich, daß Blutserum die hämolytische Wirkung verschiedener Seifen genau in derselben Weise wie die der Extraktlysine hindert.

¹⁾ Noguchi, On certain thermostabile venom activators. Journ. of Exper. Med. 8, 1906.

²⁾ Korschun und Morgenroth. Über die hämolytische Eigenschaft von Organextrakten. Berl. klin. Wochenschr. 39, 870, 1902.

Die folgende Tabelle zeigt die Resultate:

Tabelle II.

2 cem einer 5proz. Suspension von Hundekörperchen.

Serum- menge	0,1 cem 0,1proz. Natriumoleat- lösung		0,2 cem 0,1proz. Magnesium- oleatlösung	
	Hundeserum	Rinderserum	Hundeserum	Rinderserum
0,1	keine Hämolyse	keine Hämolyse	keine Hämolyse	keine Hämolyse
0,07	" "	" "	" "	" "
0,05	" "	" "	" "	" "
0,04	starke Hämolyse	schwache Hämolyse	schwache Hämolyse	" "
0,03	teilw. Hämolyse	" Hämolyse	" Hämolyse	" "
0,02	c. Hämolyse	" "	" "	" "
0,01	" "	" "	" "	" "
0	" "	" "	" "	" "

Aus den obigen Versuchen geht hervor, daß Ölsäureseifen leicht durch kleine Mengen verschiedener Normalsera inaktiviert werden. Diese Antiseifeneigenschaft des Serums ist nicht spezifisch. Ähnliche Resultate erhielt man mit den anderen organischen Öl- und Stearinseifen. Die Menge des zur Inaktivierung einer gegebenen Art von Seife erforderlichen Serums ist direkt proportional zur hämolytischen Kraft der letzteren. Die Blutkörperchen von größerem Widerstand werden durch eine kleinere Menge Serum geschützt.

Die Neutralisierung der vorhandenen Alkalinität des Serums oder halbstündiges Erwärmen auf 56° C ändert die antihämolytische Wirkung des Serums auf Seifen nicht.

Die komplementäre Funktion der mit Serum versetzten Seifen.

Die hämolytische Wirkung von Extraktlysinen und Seifen wird leicht durch adäquate Mengen nichtspezifischen Serums aufgehoben. Welches wird aber das Resultat sein, wenn wir in eine solche inaktive Mischung vorher sensibilisierte Blutkörperchen einführen, und welche Wirkung wird die Hinzugabe einer kleinen Menge stark spezifischen Serums ohne Komplement ausüben?

Für diesen Versuch wurden gewaschene Rinder- und Meer-schweinchenblutkörperchen in entsprechendem spezifischen Immunserum, das vorher auf 51° C erhitzt worden war, um die komplementäre Wirkung des Serums aufzuheben, sensibilisiert. Das Immunserum für Rinderblutkörper erhielt man von einem Schafe, das mit Ochsenblut vorbehandelt worden war und die

Stärke von 0,001 ccm = vollkommene Hämolyse von 2 ccm fünfprozentiger Suspension gewaschener Rinderblutkörper bei Gegenwart von 0,1 ccm Meerschweinchenkomplement besaß. Das spezifische Serum für die Meerschweinchenblutkörperchen wurde von einem immunisierten Kaninchen genommen. Die minimale vollständige hämolytische Dosis betrug 0,02 ccm. Die Sensibilisierung wurde durch Digerieren von gewaschenen Blutkörperchen in spezifischem, inaktivem Serum im Verhältnis von 10 zu 100 ausgeführt, indem man die Emulsion eine Stunde lang bei 38° C ließ. Nach dieser Behandlung wurden die Blutkörperchen in Salzlösung gewaschen, um das Serum zu entfernen. Das folgende Experiment gibt eines der Beispiele wieder, bei welchen Ölseifen als Komplemente dienten.

Tabelle III.

	Kontrollprobe mit physiolog. NaCl-Lsg.		Meerschweinchen-serum (Komplement) 0,1 ccm		Meerschweinchen-serum, auf 51° erhitzt, 0,1 ccm		0,15 ccm $\frac{1}{100}$ Na-Oleat u. 0,1 ccm auf 51° erhitztes Meerschweinchen-serum		0,15 ccm $\frac{1}{100}$ Ammonium-oleat und 0,1 ccm auf 51° erh. Meerschweinchen-serum		0,15 ccm $\frac{1}{100}$ Neurinoleat und 0,1 ccm auf 51° erhitzt. Meerschweinchen-serum	
	Normale Zellen	Sensib. Zellen	Normale Zellen	Sensib. Zellen	Normale Zellen	Sensib. Zellen	Normale Zellen	Sensib. Zellen	Normale Zellen	Sensib. Zellen	Normale Zellen	Sensib. Zellen
a) 2 ccm einer 5proz. Aufschwemmung von Ochsenblutkörperchen	keine Häm.	keine Häm.	keine Häm.	c. Häm. (sofort)	keine Häm.	keine Häm.	keine Häm.	c. Häm. (langsam)	keine Häm.	c. Häm. (langsam)	keine Häm.	c. Häm. (langsam)
b) 2 ccm einer 5proz. Aufschwemmung von Meerschweinchenblutkörperchen	keine Häm.	keine Häm.	keine Häm.	c. Häm. (sofort)	keine Häm.	keine Häm.	keine Häm.	c. Häm. (langsam)	keine Häm.	c. Häm. (langsam)	keine Häm.	c. Häm. (langsam)

Gewisse Unterschiede machen sich in der Geschwindigkeit der komplementären Wirkung der genuinen und der künstlichen Komplemente bemerkbar; die letzteren wirken nämlich viel langsamer als die ersteren. Man findet noch eine andere Eigentümlichkeit, wenn man Seifen als Komplemente verwendet. Während genuines Komplement nicht leicht durch einen Überschuß inaktiven Immunserums beeinflußt wird — es sei denn, daß es eine große Menge Präcipitin enthält — wird die Wirkung der Seifenserumkomplemente vollständig aufgehoben, sobald die Menge

des Immunserums eine gewisse Grenze überschreitet. Natürlich kann eine solche Inaktivierung infolge überschüssigen Serums durch Erhöhung der Seifenmengen vermieden werden.

Im folgenden Experiment wurde das spezifische Serum nicht entfernt, sondern in der Mischung belassen.

Tabelle IV.

	0,3 ccm Anti-Rinder Serum vom Schaf (auf 51° erhitzt)		1,3 ccm Anti-Meerschwein- chenserum vom Kaninchen (auf 51° erhitzt)	
	Kontrollprobe mit Salzlösung	plus 0,4 ccm 1/100-n-Neurin- oleat	Kontrollprobe mit Salzlösung	plus 0,4 ccm 1/100-n-Neurin- oleat
2 ccm 5proz. Sus- pension v. Rinder- blutkörperchen	keine Häm.	c. Hämolyse (langsam)	keine Häm.	keine Häm.
2 ccm 5proz. Sus- pension von Meer- schweinchenblut- körperchen	keine Häm.	Spur Häm. (langsam)	keine Häm.	c. Hämolyse (langsam)

Bei diesem Versuch finden wir, daß die empfindlicheren Meerschweinchenblutkörperchen durch Anti-Rinderimmunserum besser geschützt werden als durch Rinder Serum, und dies zeigt deutlich den Einfluß der in der Mischung vorhandenen Immunkörper.

Eine ähnliche Reihe von Experimenten wurde mit Normalamboceptoren angestellt, und zwar mit ungefähr demselben Ergebnis wie mit spezifischem Serum.

Tabelle V.

	1 ccm Hundeserum (50°)		1 ccm Rinder Serum (50°)		1 ccm Kaninchen- serum (50°)	
	Kontrolle mit Salz- lösung	0,2 ccm 1/100-n-Am- monium- oleat	Kontrolle mit Salz- lösung	0,2 ccm 1/100-n-Am- monium- oleat	Kontrolle mit Salz- lösung	0,2 ccm 1/100-n-Am- monium- oleat
2 ccm 5proz. Hunde- blutkörperchen	keine Hämolyse	keine Hämolyse	keine Hämolyse	Spur von Hämolyse	keine Hämolyse	keine Hämolyse
2 ccm 5proz. Rinder- blutkörperchen	keine Hämolyse	schwache Hämolyse	keine Hämolyse	keine Hämolyse	keine Hämolyse	Spur von Hämolyse
2 ccm 5proz. Kanin- chenblutkörperchen	keine Hämolyse	c. Häm- olyse	keine Hämolyse	c. Häm- olyse	keine Hämolyse	keine Hämolyse
2 ccm 5proz. Schaf- blutkörperchen	keine Hämolyse	c. Häm- olyse	keine Hämolyse	Spur von Hämolyse	keine Hämolyse	starke Hämolyse

Noch durch ein anderes Experiment wurde die Empfänglichkeit der sensibilisierten Blutkörperchen für die hämolytische Wirkung von Seifen bewiesen. Gewaschene Schafblutkörperchen wurden mit einem spezifischen Serum von einer immunisierten Ziege sensibilisiert und dann der Wirkung von Ammoniumoleatlösungen ausgesetzt. Es ergab sich, daß eine Dosis, die nicht ausreichend war, um bei den normalen (nichtsensibilisierten) Blutkörperchen Hämolyse hervorzubringen, eine fast vollständige Hämolyse der sensibilisierten Blutkörperchen hervorrief. In einer kürzlich erschienenen Arbeit¹⁾ war ich imstande, eine nahe Beziehung zwischen der Empfänglichkeit verschiedener Blutarten für Gifte und ihrem Fettsäuregehalt festzustellen, derart, daß der Grad der Empfindlichkeit der Blutkörper mit der Menge der in ihnen enthaltenen Fettsäuren (hauptsächlich Ölsäure) zunimmt. Lecithin scheint in diesen Blutkörpern in einer von Giften unangreifbaren Form vorhanden zu sein, da seine Anwesenheit in den Zellen durch das Gift nicht nachweisbar ist, obgleich es bei einer Alkoholextraktion in fast gleicher Menge ohne Unterschied zwischen Empfänglichkeit und Unempfindlichkeit der Blutkörperchen abgegeben wird. Ich fand wenigstens zwei verschiedene Klassen von Giftaktivatoren im frischen Serum. Die eine war wärmebeständig, die andere thermolabil. Die thermostabilen Aktivatoren waren manchmal Eiweißverbindungen des Lecithins, die Chabriés²⁻³⁾ Albumon ähnelten, und manchmal Ölsäure oder möglicherweise noch einige andere Fettsäuren. Über die Natur der thermolabilen Aktivatoren blieb die Wahl zwischen sogenanntem Komplement und gewissen Seifen. Tatsächlich ist es fast unmöglich, irgend einen Unterschied zwischen den Komplementen und den serumisierten Seifen festzustellen, die als Giftkatalysatoren wirken, da sie beide langsame Hämolyse von natürlich unempfindlichen, mit Gift behandelten Blutkörperchen herbeiführen und ihre Wirkung durch halbstündiges Erwärmen auf 56° C einbüßen. Temperatursteigerung über 60° C ändert die Zusammensetzung des Serums.

1) Noguchi, On extracellular and intracellular venom activators of the blood with especial reference to lecithin and fatty acids and their compounds. Journ. of Exp. Med. 9, 436, 1907.

2) Chabrié, Compt. rend. de l'Acad. d. Sc. 113, 557, 1891.

3) Howell, Amer. Journ. of Physiol. 17, 280, 1906—7.

In der folgenden Tabelle sind die Werte verschiedener Seifen als Giftaktivatoren angegeben. Die Rinderblutkörperchen waren der Wirkung von Seifen bei An- und Abwesenheit von Cobragift ausgesetzt, deren eigene hämolytische Kraft durch eine genügende Menge Normalserum aufgehoben worden war. Frisches Normalrinderserum enthält gar keine Giftaktivatoren. Die Blutkörperchen wurden als 5prozentige Aufschwemmung in 3 ccm einer 0,9prozentigen Salzlösung angewendet, in der die angegebenen Mengen Seifen, Serum und Gift enthalten waren.

Tabelle VI.

0,5 ccm normales Rinderserum pro Gläschen (zur Inaktivierung der Seifen)									
	0,2 ccm 1proz. Natriumoleat	0,2 ccm 1proz. Ammoniumoleat	0,2 ccm 1proz. Neurinoleat	0,2 ccm 1proz. Magnoleat	0,5 ccm 1proz. Calciumoleat	0,2 ccm 1proz. Natriumstearat	0,2 ccm 1proz. Magnesiumstearat	0,5 ccm 1proz. Calciumstearat	Kontrollprobe ohne Seife
0,5 ccm 1proz. Cobragiftlösung	c. Häm. (verzögert)	c. Häm.	c. Häm.	c. Häm.	c. Häm. stark verzög.	c. Häm. stark verzög.	starke Häm.	schw. Häm.	keine H.
Ohne Cobragift	keine H.	keine H.	keine H.	keine H.	keine H.	keine H.	keine H.	keine H.	keine H.

Die durch Hinzufügen von Serum angenommenen Eigenschaften der Seifen.

Verschiedene Ölsäureseifen werden vollständig unwirksam, wenn man sie mit ausreichenden Mengen Serum vermischt. Dieser inaktive Zustand der Seifen geht in einen aktiven über, sobald spezifische Intermediärkörper auf die Blutkörperchen wirken können, oder wenn die Blutkörper in geeigneter Weise sensibilisiert worden sind. Abgesehen vom langsameren Fortschreiten der Hämolyse als bei den genuinen Komplementen und der höheren Empfänglichkeit für die antikomplementäre Wirkung des Serums könnte man die serumisierten Seifen als Komplemente im Sinne unserer gegenwärtigen biologischen Terminologie auffassen.

Aber wie weit diese serumisierten Seifen dem genuinen Serumkomplement ähnlich sind, bleibt noch auf anderem, sicherem Wege zu erforschen.

1. Spontanes Verschwinden der komplementären Eigenschaft der serumisierten Seifen: Eine Mischung von

Ammoniumoleat und von auf 50° C erhitztem Meerschweinchen-serum wurde in entsprechendem Verhältnis als Komplement für sensibilisierte Meerschweinchenblutkörperchen bei Zimmertemperatur stehen gelassen. Nicht erhitztes Meerschweinchen-serum wurde genau denselben Bedingungen wie die serumisierte Seifenmischung ausgesetzt. Nach zwei Wochen wurde ersteres auf seine komplementäre Eigenschaft hin geprüft, und man fand, daß es seine Aktivität während der oben erwähnten Zeitdauer verloren hatte. Das Kontrollserum war ebenfalls in dieser Hinsicht inaktiv. Es wurden keine Versuche angestellt, um die kürzeste zur Inaktivierung der Mischung erforderliche Zeitdauer festzustellen.

2. Inaktivierung der serumisierten Seifen bei 50° C: Alle löslichen Seifen, besonders die Ölsäureseifen verlieren, wenn sie mit Serum vermischt sind, bei halbstündiger Erhitzung auf 56° C ihre komplementäre Wirkung. In dieser Hinsicht zeigen die serumisierten Seifenkomplemente eine auffallende Ähnlichkeit mit Serumkomplementen.

3. Der Einfluß niedriger Temperatur auf die komplementäre Wirkung serumisierter Seifen: Wie die Serumkomplemente sind die serumisierten Seifen in bezug auf ihre komplementäre Wirkung bei 0° C inaktiv.

4. Die Wirkung verschiedener Säuren, Alkalien und Salze auf serumisierte Seifen: Wie bei den Serumkomplementen wird die komplementäre Wirkung der serumisierten Seifen durch verschiedene Säuren, mit Ausnahme der höheren Fett- und Acrylsäuren, aufgehoben. Einige Calcium- und Bariumsalze sind kräftig antihämolysisch. Bindung der Säuren durch entsprechende Alkalien stellt die Aktivität wieder her, und die inaktiven Seifen werden durch entsprechende Salze wieder wirksam gemacht. Die Inaktivierung serumisierter Seifen durch Säuren geschieht durch die Abspaltung von Fettsäuren, mit denen das Serum inaktive Verbindungen bildet. In einem eiweißfreien Medium erfolgt durch Säuren keine Inaktivierung, da in diesem Falle die freigewordenen Fettsäuren eine kräftige hämolysische Wirkung ausüben. Wie Serumkomplement werden serumisierte Seifen durch Calciumcarbonat nicht sehr angegriffen, während in einer eiweißfreien Flüssigkeit allmählich unlösliche Calciumseifen gebildet werden und die hämolysische Kraft infolgedessen verloren geht oder sinkt. Die Wirkung schwacher Alkalien auf Serum-

komplemente ist eine hemmende, und dasselbe gilt von den serumisierten Seifen, obwohl der störende Einfluß bei letzteren weniger ausgesprochen ist. Bei einem eiweißfreien Medium beschleunigt das Hinzufügen von Alkali den durch Seifen verursachten Eintritt der Hämolyse. Es kann behauptet werden, daß die Extraktlysine sich in bezug auf obige Reaktionen von den Seifenlösungen nicht unterscheiden.

5. Inaktivierung der serumisierenden Seifen durch Zellelemente: Ein vollständiger Verlust der komplementären Eigenschaft serumisierter Seifen wurde nachgewiesen, wenn die Mischungen zwei Stunden lang mit Hefezellen, Leber-, Nieren- und Milzzellen, *B. anthracis* und *B. typhi* bei 37° C behandelt waren. Cholesterin verzögert in großer Menge die komplementäre Wirkung, aber sein Einfluß ist unsicher. Sogar in einer eiweißfreien Lösung wird die hämolytische Kraft der Seife mehr oder minder durch Berührung mit den obenerwähnten Zellarten geschwächt.

6. Der hemmende Einfluß von „Schutzstoffen“: Aus Serum, Leber, Niere, Milz, *Diplococcus intracellularis* und Gonokokken gewonnene Schutzstoffe verhindern die Hämolyse durch Seifen.

7. Einfluß beschleunigter Oxydation durch photodynamische Substanzen. Direktes Sonnenlicht zerstörte bei Gegenwart von 0,025% reinem Eosin sowohl die komplementäre Wirkung der serumisierten Seifen als genuines Serumkomplement innerhalb 6 Stunden. Die Kontrollproben ohne diesen Farbstoffzusatz behielten noch einen erheblichen Teil ihrer Aktivität nach dem Exponieren, während die Kontrollproben mit Farbstoff im Dunkeln keine bemerkenswerte Abnahme aufweisen. Eine eiweißfreie Seifenlösung erfährt, wenn sie mit reinem Eosin vermischt und dem Sonnenlicht mehrere Tage lang ausgesetzt wird, nur eine leichte Beeinträchtigung.

B. Bactericide Versuche.

Ogleich die Versuche noch in mancher Beziehung unvollständig sind, möchte ich doch an dieser Stelle einige vorläufige Angaben über die bactericide Eigenschaft gewisser Ölsäureseifen machen. Während viele Untersuchungen die Ansicht stützen, daß das die Bakteriolyse herbeiführende Komplement sich von dem die Hämolyse bewirkenden unterscheidet, ist das Serum-

komplement der Absorption durch sensibilisierte Bakterien ebenso wie durch sensibilisierte Blutkörperchen unterworfen. Die Bildung spezifischer Präcipitate verursacht das Verschwinden des Komplements in vivo und in vitro. Der Verlust des Komplements in vivo wird durch die Abwesenheit von Bakteriolyse¹⁾ und Hämolyse²⁻³⁾ bewiesen. Ältere Experimente geben an, daß Leukocytenextrakte bakteriolysisch sein können, ohne zu gleicher Zeit hämolytisch zu sein. Aber man muß die Tatsache betonen, daß kein Beweis dafür existiert, daß der Unterschied durch Mangel an Komplement verursacht war. Es ist nicht unwahrscheinlich, daß die Extrakte frei von hämolytischen Amboceptoren, aber nicht von Komplement waren. Die Erscheinungen sprechen heutzutage mehr für die Ansicht, daß sowohl bakteriolysische als hämolytische Komplemente einander vertreten, wenn nicht identisch sind.

Die Resultate der oben angegebenen hämolytischen Experimente veranlaßten mich zu Nachforschungen darüber, ob die serumisierten Seifen nicht instande sind, normale und spezifisch bakteriolysische Intermediärkörper zu aktivieren.

Die normalen Sera von Hund, Kaninchen und weißer Ratte wurden wechselseitig gegen *B. anthracis* und *B. typhosus* geprüft, und zwei Immun-Sera gegen *B. typhosus* und *B. dysenteriae*.

Die angewandte Technik ist dieselbe, die Neisser und Wechsberg⁴⁾ angegeben haben; sie bestand darin, daß $\frac{1}{5000}$ ccm einer 24stündigen Bouillonkultur der Einwirkung der Mischung ausgesetzt und dann beobachtet wurde.

Bei *B. anthracis* wurde eine Emulsion einer 24 Stunden alten Agarkultur in 0,9proz. Salzlösung bei 16° C hergestellt. Die Schätzung der bactericiden Kraft wurde bewerkstelligt, indem man die Zahl der Kolonien zählte, die auf mit fünf Ösen der Mischung geimpften Agarboden nach sechsstündigem Verweilen

¹⁾ Pfeiffer und Moreschi, Über scheinbare antikomplementäre und Antiamboceptorwirkungen präcipitirender Sera im Tierkörper. Berl. klin. Wochenschr. 43, 33, 1906.

²⁾ Ehrlich und Morgenroth, loc. cit.

³⁾ Fleischmann und Michaelis, Über experimentell in vivo erzeugten Komplementschwund. Med. Klinik 2, 21, 1906.

⁴⁾ Neisser und Wechsberg, Über die Wirkungsweise bactericider Sera. Münch. med. Wochenschr. 43, 697, 1901.

bei 37° C gewachsen waren. Das Gesamtvolumen betrug gleichmäßig 2,5 ccm und jedes Röhrchen enthielt drei Tropfen Bouillon, um das Verhungern der Bakterien zu verhüten.

Die Tabellen VII und VIII zeigen die Resultate mit normalen Seris. Die Menge des in einem Röhrchen enthaltenen Serums betrug 0,5 ccm.

Tabelle VII.

B. typhosus und normales Serum.

	0,5 ccm frisches Serum	0,5 ccm auf 60° erhitztes Serum					Seifenlösung ohne Serum			Kontrollprobe mit reiner Salzlösung
		Ohne Kom- plement	+ 0,1 ccm Meerschwein- chenserum	+ 0,5 ccm $\frac{1}{100}$ n-Ammo- niumoleat	+ 0,5 ccm $\frac{1}{100}$ n-Neurin- oleat	+ 0,5 ccm $\frac{1}{100}$ n-Natri- umoleat	+ 0,5 ccm $\frac{1}{100}$ n-Ammo- niumoleat	+ 0,5 ccm $\frac{1}{100}$ n-Neurin- oleate	+ 0,5 ccm $\frac{1}{100}$ n-Natri- umoleat	
Hunde- serum	wenige Hundrt.	einige Tausnd.	einige Hundrt.	einige Hundrt.	einige Hundrt.	einige Hundrt.	wenige Hundrt.	wenige Hundrt.	wenige Hundrt.	einige Zehn- tausend
Kanin- chen- serum	wenige Hundrt.	un- zählige	einige Tausnd.	einige Hundrt.	einige Hundrt.	einige Hundrt.	wenige Hundrt.	wenige Hundrt.	wenige Hundrt.	un- zählige
Ratten- serum	wenige Hundrt.	einige Hundrt.	einige Hundrt.	einige Hundrt.	einige Hundrt.	einige Hundrt.	wenige Hundrt.	wenige Hundrt.	wenige Hundrt.	un- zählige

Tabelle VIII.

B. anthracis und normales Serum.

	0,5 ccm frisches Serum	0,5 ccm auf 60° erhitztes Serum					Seifenlösung ohne Serum.			Kontrolle mit reiner Salzlösung
		Ohne Kom- plement	+ 0,1 ccm Meerschwein- chenserum	+ 0,5 ccm ¹ / ₁₀₀ n-Ammo- niumoleat	+ 0,5 ccm ¹ / ₁₀₀ n-Neu- rinoleat	+ 0,5 ccm ¹ / ₁₀₀ n-Natri- umoleat	0,5 ccm ¹ / ₁₀₀ n- Ammonium- oleat	0,5 ccm ¹ / ₁₀₀ n- Natriumoleat	0,5 ccm ¹ / ₁₀₀ n- Neurinoleat	
Hunde- serum	wenige Tausd.	einige Tausd.	wenige Tausd.	wenige Hundrt.	wenige Hundrt.	wenige Hundrt.	0	0	0	einige Tausd.
Kanin- chen- serum	wenige Hundrt.	wenige Tausd.	wenige Hundrt.	ungef. Hundrt.	ungef. Hundrt.	ungef. Hundrt.	0	0	0	einige Tausd.
Ratten- serum	0	einige Tausd.	beinahe Hundrt.	wenige Zehn	wenige Zehn	wenige Zehn	0	0	0	einige Tausd.

B. typhosus wurde durch keines der drei Sera in frischem Zustand vollständig getötet, aber die Vermehrung wurde verhindert, wie man aus der Zahl der auf den „Salzkontroll“-Böden erscheinenden Kolonien ersehen kann. Erhitzte Sera (auf 60° C)

bildeten gute Nährböden, und daher zeigte sich reichliches Wachstum auf ihnen. Reaktivierung des erhitzten Serums durch Hinzufügen von 0,1 ccm frischen Meerschweinchenserums pro Gläschen, war teilweise von Erfolg. Andererseits stellte das Hinzufügen von Oleat-Seifen, einschließlich Ammonium-, Neurin- und Natrium-Seifen, in einer Menge von 0,5 ccm $\frac{1}{100}$ -n pro Gläschen, etwa denselben Grad antibakterieller Wirkung dar, wie die des frischen Normalserums. Es ergab sich jedoch, daß diese Seifen in einem serumfreien Medium stärker bactericid sind. *B. anthracis* wurde in einem serumfreien Medium, das 0,5 ccm von $\frac{1}{100}$ n-Seifenlösung enthielt, vollständig zerstört, während in einer seifenfreien Flüssigkeit eine starke Vermehrung erfolgte. Von den drei Normal-Seris zerstörte das der weißen Ratte den Anthrax-Bacillus am kräftigsten und das des Hundes war fast inaktiv. Das durch einstündiges Erhitzen auf 60° C inaktiv gewordene Rattenserum wird durch Hinzufügen von 0,1 ccm frischem Meerschweinchen- oder Kaninchenserum wieder aktiv. Man fand, daß Mischung von inaktiviertem Rattenserum (60° C) und von Seifenlösung eine Verminderung der der Seife innewohnenden bactericiden Kraft ergibt, und zwar durch die Antiseifeneigenschaft des Serums und eine vom Serum vermöge der vorhandenen Seife angenommene ausgeprägte bactericide Kraft. Das erhitzte Hundeserum weist eine größere antibakterielle Kraft gegen Seifen auf, was seine Erklärung im Mangel an genügend Intermediärkörpern finden kann, um die zerstörende Kraft der einmal durch das Serum paralysierten Seifen zu beschleunigen.

Die Resultate mit Immunserum zeigen deutlicher als die mit Normalserum, daß Oleatseifen als bactericide Komplemente wirken können. Wie Tabelle IX erkennen läßt, bewirkte 0,1 ccm frischen Antityphusserums von einem immunisierten Kaninchen eine beträchtliche Hemmung des Wachstums von *B. typhosus*. Diese Hemmungswirkung verschwand bei halbstündiger Erwärmung auf 56° C und kehrte bei Hinzufügen von 0,1 ccm frischen Meerschweinchenserums zum erhitzten Serum zurück. Alle Oleatseifen sind auch antibakteriell gegen typhoide Bacillen und bewirken ausgesprochene Einschränkung ihrer Vermehrung, wenn sie in der in der Tabelle angegebenen Konzentration verwendet werden. Ihre Wirksamkeit übertraf die Kraft des in diesem Experiment benutzten frischen Immunserums. Das auffallendste Versuchs-

ergebnis ist jedoch die Wirkung der Oleatseifen auf das inaktivierte Immunserum. Man fand, daß das Vorhandensein einer kleinen Menge des inaktiven Immunserums die bactericide Kraft der Seifen gegen *B. typhosus* vermehrte. In diesem Falle scheint es sich um einen tatsächlichen Komplementamboceptor vom Lysintypus zu handeln.

Tabelle IX.

B. typhosus und antityphoides Immunserum.

0,1 cem frisches Immun- serum	0,1 cem auf 56° erhitztes Immunserum					Seitenlösung ohne Serum				Kontroll- probe mit reiner Salz- lösung
	Ohne Kom- plement	+ 0,1 cem Meerschwein- chenserum	+ 0,5 cem 1 ₁₀₀ n-Ammo- niumoleat	+ 0,5 cem 1 ₁₀₀ n-Neurin- oleat	+ 0,5 cem 1 ₁₀₀ n-Natri- umoleat	+ 0,5 cem 1 ₁₀₀ n-Ammo- niumoleat	+ 0,5 cem 1 ₁₀₀ n-Neurin- oleat	+ 0,5 cem 1 ₁₀₀ n-Natri- umoleat		
einige Hundert	un- zählige	wenige Hundert	0	0	zehn	wenige Hundert	wenige Hundert	wenige Hundert	unzählige	

Ein ähnliches, aber weniger ausgesprochenes Resultat erhielt man mit *B. dysenteriae* und einem alten Pferdeimmunserum. Bei diesem Beispiel finden wir wieder das Phänomen der teilweisen Reaktivierung eines unwirksamen Immunserums durch Hinzufügen einer gewissen Menge löslicher Oleatseifen.

Tabelle X.

B. dysenteriae und Antidysenterie-Immunserum.

0,2 cem unwirk- sames Immun- serum	0,2 cem inaktives Immunserum					Seifenlösung ohne Serum			Kontrollproben mit reiner Salz- lösung
	+ 0,1 cem Meerschwein- chenserum	+ 0,2 cem Meerschwein- chenserum	+ 0,5 cem 1 ₁₀₀ -n-Am- moniumoleat	+ 0,5 cem 1 ₁₀₀ -n-Neu- rinoleat	+ 0,5 cem 1 ₁₀₀ -n-Natri- umoleat	0,5 cem 1 ₁₀₀ -n- Ammonium- oleat	0,5 cem 1 ₁₀₀ -n- Neurinoleat	0,5 cem 1 ₁₀₀ -n- Natriumoleat	
un- zählige	einige Tausend	etwa tausend	etwa hundert	etwa hundert	etwa hundert	wenige Hundert	wenige Hundert	einige Hundert	un- zählige

Die Kontrollproben mit inaktiviertem Normalpferdeserum zeigten, daß die Mengen von 0,1 cem bis 0,2 cem Serum die den Oleatseifen innewohnende lytische Kraft bis zu einem beträchtlichen Grade verminderten.

Während die oben angegebenen Experimente zu zeigen scheinen, daß unter gewissen Bedingungen der Immunsera lös-

liche Oleatseifen als Komplemente dienen können, fand ich mehrere Fälle, bei denen das Phänomen der Reaktivierung nicht auftrat. Ich arbeitete mit einem Stamme des *Vibrio* der asiatischen Cholera und mit einem Kaninchen-Immunserum von beträchtlicher Aktivität und fand, daß das einmal durch Erhitzung auf 56° inaktivierte Serum bloß als antibactericide Substanz gegen den Einfluß der Oleatseifen wirkte. Es sei bemerkt, daß ölsaures Natrium ein sehr kräftiges Vibriocid ist. Das Hinzufügen von 0,2 ccm einer zweiprozentigen Seifenlösung zu 1 ccm einer 24 Stunden alten Bouillonkultur genügt, um die Mischung im Laufe von mehreren Stunden bei 38° C zu sterilisieren. Es ist jedoch zu hoffen, daß noch zahlreichere Versuche mit verschiedenen Abänderungen und unter wechselnden Bedingungen angestellt werden, um die Wichtigkeit und den Umfang der Bedeutung von gewissen löslichen Seifen für die Schutzmaßregeln des lebenden Organismus gegen eindringende fremde Mikroorganismen zu bestimmen.

Zwar können die Ölsäureseiten nicht alle Komplementärs-substanzen, die im Blute vorhanden sind, repräsentieren, oder sind vielleicht auch nicht die wesentlichen unter ihnen, doch verdient ihre ausgesprochene komplementäre Wirkung bei hämolytischen sowohl als bei bactericiden Intermediärkörpern mehr Aufmerksamkeit, als man ihr bisher zugewendet hat.

Zusammenfassung.

Das Blutserum hat einen Bestandteil, der als Alexin oder Komplement bekannt ist und Blutkörperchen oder Bakterien auflöst, wenn letztere in geeigneter Weise sensibilisiert sind. Die Wirkung des Komplements verschwindet, wenn das Serum alt wird oder kurze Zeit lang auf 56° C erhitzt ist. Das Schicksal des Komplements nach der Erhitzung oder Beeinträchtigung durch Alter ist nicht bekannt, aber es wird allgemein angenommen, daß es zersetzt wird. Blutserum ergibt bei Extraktion mit warmem Alkohol eine Substanz oder eine Gruppe von Substanzen mit kräftigem Lösungsvermögen. Dasselbe gilt von Leukocyten, Drüsenzellen und gewissen inneren Organen. Hinsichtlich einiger Unterschiede im lytischen Mechanismus und in der Wärmebeständigkeit zwischen Genuinserumkomplement und Extrakt-lysin war kein direkter Vergleich angestellt worden, um eine

mögliche Verwandtschaft zwischen den beiden zu entscheiden. Komplement ist bloß in Anwesenheit von Immunkörpern lytisch, während die Extraktlysine an sich wirksam sind. Die Wirkung des Komplements nimmt mit der Zeit ab und wird durch eine Temperatur von 56°C unterdrückt, wohingegen die Extraktlysine sich mit der Zeit nicht verschlechtern oder beim Kochen verschwinden. Es ist die heutige allgemeine Auffassung, daß sie zwei ganz verschiedene Körperklassen sind. Bis heute ist keine Angabe über die Rolle, welche die anderen Serumkomponenten spielen, gemacht worden. Ein Vergleich, der unter verschiedenen Bedingungen angestellt ist, hat keinen Wert, und Betrachtungen über diesen Punkt bilden den ersten Gegenstand der vorliegenden Arbeit. Ich erhielt aus Blut, Leber, Niere und Milz von Hund, Kaninchen und Rind durch längere Extraktion mit warmem Alkohol eine alkohollösliche Fraktion. Es ergab sich, daß dieser Anteil nach Befreiung von Neutralfetten, Fettsäuren, Lecithin, Cholesterin und anderen in Äther löslichen Substanzen stark hämolytisch war. Seine Wirkung ist nicht spezifisch und erfordert keine Intermediärkörper. Diese Fraktion besteht aus verschiedenen Seifen, besonders aus Ölsäureseifen, die dem Blut und den Geweben entstammen. Zugabe einer geeigneten Menge indifferenten oder nicht spezifischen Serums zu dem Extrakt vermindert seine lytische Wirkung. Aber diese Inaktivierung ist nur oberflächlich; denn die Mischung ist nicht unwirksam gegen Blutkörperchen, die mit spezifischen oder normalen Intermediärkörpern behandelt sind, noch ist sie bei Gegenwart einer entsprechenden Menge Immunserums ohne Einfluß, mit anderen Worten: diese Seifenfraktion oder das Extraktlysin erwirbt die Eigenschaft, als Komplement zu wirken. Dieses künstliche Komplement kann leicht durch einhalbstündige Erwärmung auf 56°C oder durch eine Woche langes oder längeres Stehenlassen bei Zimmertemperatur inaktiviert werden. Die komplementäre Wirkung bleibt bei 0°C aus. Wie Serumkomplement wird es inaktiv, wenn man es mit adäquaten Mengen von Erdalkalisalzen starker Säuren und jeder stärkeren Säure als Kohlensäure vermischt. Alkalien verzögern die Wirkung des Gemisches. Man findet, daß die Seifenfraktion in einer eiweißfreien Lösung durch Säuren oder Alkalien nicht inaktiviert werden kann. Ohne Eiweißgehalt kann kein Unwirksamwerden bei 56°C oder durch Alter oder bei

0° C erreicht werden. Alle diese Eigenschaften des Komplements können möglicherweise der Gegenwart von Serumproteinen zugeschrieben werden.

Meine Versuche mit reinen Präparaten verschiedener Seifen bestätigen nicht nur die obigen Befunde, sondern liefern ferner eine Erklärung für den Inaktivierungsvorgang bei den Extraktlysinen des Blutes und der Gewebe durch verschiedene Erdalkalisalze.

Ob derselbe Mechanismus für die Inaktivierung des Genuin-serum-Komplements durch diese Salze angenommen werden muß, kann nicht festgestellt werden, obgleich das Phänomen eine sehr große Ähnlichkeit zeigt. Bei diesen Versuchsreihen wurden Oleate sowohl als Stearate von Ammonium, Neurin, Natrium, Magnesium und Barium angewandt. Ölsäureseifen sind in 0,9 proz. Salzlösung löslicher als die entsprechenden Stearate. Hinsichtlich der hämolytischen Wirksamkeit kann man festsetzen, daß die Oleate fast 10mal so kräftig wie die Stearate wirken und daß alle unlöslichen Seifen fast ohne lytische Wirkung sind. Von den Oleaten ist Neurinseife die am leichtesten und Ammoniumseife die am schwersten lösliche. Ebenso wie die Seifenfraktion des Blutes oder der Gewebe werden alle diese Seifen beim Vermischen mit Serum inaktiv. Der Wert der mit Serum versetzten (serumisierten) Ölsäureseifen als Komplemente für Serum und Giftamboceptoren ist der gleiche wie der von Extraktlysinen. Die Wirkung verschiedener physikalischer und chemischer Einflüsse auf ihre Aktivität ist genau dieselbe wie bei den Extraktlysinen. Mit Bezug auf die Hämolyse ist die komplementäre Wirkung der serumisierten Oleate beträchtlich langsamer als die von genuinem Serumkomplement und wird noch mehr durch die Gegenwart von Serumbestandteilen verzögert. Diese Unterschiede zwischen künstlichen und natürlichen Komplementen sind nicht so ausgesprochen, wenn Gift als Sensibilisator gebraucht wird.

Die Komplementäreigenschaft der Seifenserummischung und des Serumkomplements wird leicht durch photodynamische Substanzen zerstört.

Die eiweißfreien Lösungen verschiedener Ölsäureseifen sind, wenn sie in der Konzentration von $\frac{1}{100}$ bis zu $\frac{1}{500}$ -n angewendet werden, in hohem Maße bactericid. Es ist festgestellt, daß *B. anthracis* und *Vibrio cholerae* für die Wirkung von Seifen

empfindlicher sind als *B. typhosus* und *B. dysenteriae*. Die bactericide Eigenschaft der Seifen wird durch Mischung mit Serum stark herabgesetzt. Während inaktives Normalserum die bactericide Kraft bis zu einem gewissen Grade einschränken kann, kommt es oft vor, daß die Gegenwart von Seifen im Serum letzterem eine mehr oder minder bactericide Kraft verleiht, die wohl als eine Art von Reaktivierungserscheinung aufgefaßt werden kann. In diesem Falle handelt es sich indessen um ein Phänomen von chemischer Zerstörung. Die Experimente mit Immunserum ergaben andererseits ein etwas abweichendes Ergebnis. Unter gewissen Bedingungen beobachtete man, daß die Mischung von Ölsäureseifen und inaktivierten Immunseris eine vollkommenere Zerstörung der Bakterien hervorbrachte als Seifen allein. Es kann sein, daß diese Erscheinung in die Kategorie der chemischen Desinfektion fällt, die Tatsache jedenfalls, daß die bakterientötende Kraft der Seifen durch die Anwesenheit spezifischer Immunkörper stark beschleunigt wurde, ist von besonderem biochemischen Interesse.

Abgesehen davon, daß gewisse lösliche Seifen aus Blut und Organen durch Behandlung mit warmem Alkohol extrahiert werden können und daß sie beträchtliche lytische Kraft besitzen, sowie im Gemisch mit Serum fast jede Eigenschaft von sogenanntem Serumkomplement annehmen und daß alle die Erscheinungen durch Anwendung chemisch reiner Seifen wiedergegeben werden können, kann man keine Schlüsse in bezug auf die Identität serumisierter Seifen und genuiner Serumkomplemente ziehen. Die im Blute und in der Lymphe enthaltenen Seifen- und Fettsäuremengen bilden die Grundlage für die Meinung, daß ein gewisser Teil der Schutzkraft des Organismus diesen Seifensubstanzen zuzuschreiben ist.

Notiz über die Pikrolonate einiger Nucleinbasen.

Von
P. A. Levene.

(Aus dem Rockefeller Institute for Medical Research, N. Y.)

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Wie bekannt, kommen bei der Analyse der Spaltungsprodukte der Nucleoproteine die „Hexon“- und die Nucleinbasen in denselben Fraktionen vor. Da nun Stendel¹⁾ und Otori²⁾ beobachtet haben, daß die erste mit Pikrolonsäure schwerlösliche Verbindungen bildet, war es von Wichtigkeit, auch das Verhalten der anderen Basen zu diesem Reagens zu untersuchen. Die Basen, welche mit den Hexonbasen mit niedergeschlagen werden können, sind Adenin, Guanin, Cytosin, und es wurde versucht, die Pikrolonate derselben darzustellen.

Adenin. Adeninsulfat wurde in Lösung gebracht, und zu dieser wurde eine konzentrierte alkoholische Pikrolonsäurelösung zugesetzt so lange, bis sich ein Niederschlag bildete. Das Pikrolonat wurde aus einer großen Menge heißen Wassers umkristallisiert. Die Substanz besaß einen S. P. = 265° C.

0,1015 g der Substanz gaben 28,2 ccm Stickstoff (über 50% KOH) bei T = 26,20 und P = 766.

Für $C_5H_5N_5 \cdot C_{10}H_8N_4O_5$

Berechnet	Gefunden
N = 31,90%	32,03%

Guanin. Freies Guanin wurde in einer genügenden Menge Normal-Natronlauge Lösung aufgelöst und mit Pikrolonsäure behandelt. Es bildete sich ein voluminöser Niederschlag, der aus Wasser umkristallisiert und analysiert war.

¹⁾ Zeitschr. f. physiol. Chemie 37, 219, 1902.

²⁾ Zeitschr. f. physiol. Chemie 43. 305.

2 P. A. Levene: Notiz über die Pikrolonate einiger Nucleinbasen.

0,1500 g der Substanz gaben 35,4 ccm Stickstoff (über 50% KOH) bei $T = 27,5$ und $P = 752$.

Für $C_5H_5N_5O \cdot 2 C_{10}H_8N_4O_5$

Berechnet	Gefunden
N = 26,64%	26,98%

Cytosin. Das Sulfat wurde in Wasser aufgelöst und mit Pikrolonsäure wie oben behandelt. Das Pikrolonat wurde aus Wasser umkristallisiert.

0,1909 g der Substanz gaben 43,4 ccm Stickstoff (über 59% KOH) bei $T = 24,5$ und $P = 760$.

Für $C_4H_5N_3 \cdot C_{10}H_8N_4O_5$

Berechnet	Gefunden
N = 26,13%	26,07%

PERISTALTIC RUSH.

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INTRODUCTION.

SOME writers on the motor phenomena of the intestinal tract distinguish at present three types of movement in the small gut. (1) Pendular movements: rhythmical, swaying motions, which apparently contribute but little to the forward expedition of the intestinal contents. According to Bayliss and Starling, and to Cannon, the pendular movements are chiefly concerned in the thorough mixing of the food with digestive fluids; they are the essential factors in the "rhythmic segmentation" of the intestinal contents observed by Cannon. (2) Peristaltic movements, which consist in a contraction of the gut above a food mass and a relaxation below it; they are chiefly concerned in carrying the food through the intestines in the aboral direction. The progress is very slow, 1 cm. in two to ten minutes in a fasting state and in thirty to forty seconds after a meal (Cash). The peristaltic waves never travel far, the same wave never running through the entire length of the intestines. (3) "Rollbewegungen," a fast, running movement extending over the whole or a large section of the small intestines. It is this motor phenomenon we intend to deal with in the present paper.

HISTORICAL.

This phenomenon was first observed and described by van Braam Houkgeest¹ with Sanders-Ezn. The observations were made on rabbits whose abdomen was opened and the intestines observed while these parts of the animal were submersed in a warm saline bath.

¹ HOUKGEEST, VAN BRAAM: PFLÜGER'S Archiv für die gesammte Physiologie, 1872, vi, p. 266.

When the animal was killed by suffocation or by inhalation of CO₂, a few seconds after the convulsions attending asphyxia ceased, a strong peristaltic contraction set in, either at the pylorus or a little distance away, which energetically and rapidly drove the contents before it. This rapid peristaltic wave ran without interruption to the cæcum, especially when the duodenum was strongly filled with contents. Sometimes another wave followed which originated in the ileum, and which also ran down in the same manner to the cæcum. Mostly, however, the duodenal wave was the only one which was observed. When the duodenum was less filled, or when the animal was already for some time in the saline bath, the peristaltic wave did not reach the cæcum, but stopped at the lower end of the jejunum or at the upper end of the ileum; in the latter case another similar wave arose later at some part of the ileum which carried the contents rapidly to the cæcum. None of the waves ever continued their course in the cæcum.

The rapid run of the peristaltic wave through the small intestine gave the coils the appearance of turning wheels; the movements were therefore designated by the investigator as "*Rollbewegungen*." At first in the protocols, but later for lack of a better expression, van Braam Houkgeest accepted this term in the final presentation of his results. Most of the subsequent writers have adopted this term for the description of this special form of intestinal peristalsis. Van Braam Houkgeest termed them in his protocols sometimes also "*post mortem movements*" on account of their occurrence only after death of the animal. Only in one case did he succeed in reviving the animal by prolonged artificial respiration after the *Rollbewegungen* had made their appearance. Later on van Braam Houkgeest was led to believe that under certain conditions he was able to produce the *Rollbewegung* also while the animal was alive. The discussion of this, however, will be deferred until later.

Observations of a similar character were also made by Engelman¹ (with van Brakel) about one year previous to the publication of van Braam Houkgeest. In a cat, which was killed by chloroform, and whose intestines were examined about half an hour after death, a mechanical stimulus applied to some part of the intestines caused a strong local constriction which ran down as a peristaltic wave to the cæcum with a rapidity of about 4 cm. in a second; upward the contraction ran as an antiperistaltic wave, stopping at

¹ ENGELMANN: *Archiv für die gesammte Physiologie*, 1871, iv, p. 33.

the stomach. In animals which were killed by exsanguination (rabbits, dogs, and cats) spontaneous movements which originated in the duodenum were seen to run down at times to the ileo-cæcal valve; mostly, however, the rapid wave stopped at the ileum.

In the experiments of van Braam Houkgeest, as well as in those of Engelmann, these movements were seen to occur after death; they were characterized by the rapid propagation of the peristaltic wave; sometimes the wave ran through the entire small intestine without stopping.

Nothnagel¹ later described Rollbewegung, which he studied in the living rabbit. He describes it as a violent peristalsis running rapidly over some 20 cm. of the small intestine and coming suddenly to a standstill, as he suggests, by some inhibitory influence. He has never seen the wave extending over the entire length of the small intestine. Nothnagel believes that the distention of the intestines by liquid and gas is the cause of these Rollbewegungen, but asserts at the same time that observation of these movements had to be confined to their accidental occurrence, and that it was impossible to produce the Rollbewegungen at will.

Bokai,² on the other hand, stated that the presence of CO₂, CH₄, or H₂S in the intestines will cause "rollende Bewegungen." These movements, when once started, are not propagated to a distant section of the intestines where these gases are absent. The gases produce violent but only local contractions. The presence of oxygen in the lumen of the intestines stops these movements; nitrogen and hydrogen exert no effect.

From the writings of Mall,³ Cannon,⁴ and others it appears that the "rollende Bewegungen" of Bokai are considered as identical with the Rollbewegungen of van Braam Houkgeest.

Cannon describes the Rollbewegungen "as a rapid movement sweeping the food without pause through several turns of the gut," and "is frequently seen when the food is carried on from the duodenum"; and states "that it may readily be produced in other parts of the small intestine by giving an enema of soapsuds."

¹ NOTHNAGEL: Beiträge zur Physiologie und Pathologie des Darms, Berlin, 1884. See also NOTHNAGEL'S Handbuch der speziellen Pathologie und Therapie, Die Darmbewegungen, 1898, xvii, p. 2.

² BOKAI: Archiv für experimentelle Pathologie und Therapie, 1887, xxiii, p. 209.

³ MALL: Johns Hopkins Hospital reports, 1896, i, p. 51.

⁴ CANNON: This journal, 1902, vi, p. 251.

We shall return now to a few statements of van Braam Houkgeest. In the first place, he states that the *Rollbewegungen* did not occur post mortem if both vagi were previously cut; only irregular *Rollbewegungen* and constrictions appeared in various sections of the small intestines. If however at the onset of these irregular contractions the peripheral ends of the cut vagi were stimulated, regular *Rollbewegungen* then ran through the entire small intestine. Furthermore, if in a living rabbit both splanchnics were cut, then stimulation of the peripheral end of either vagus would cause first a strong constriction of the median part of the stomach which was followed soon by a "true *Rollbewegung*," which in most cases ran through the entire small intestine. In strong animals such results could be obtained repeatedly by repeated stimulation of the peripheral end of either vagus. Van Braam Houkgeest believes that the *Rollbewegungen* produced by the stimulation of the vagi, after cutting the splanchnics, are identical with the post mortem *Rollbewegungen*.

In summing up this review we find that Engelmann as well as van Braam Houkgeest have observed in dead animals *Rollbewegungen* of spontaneous origin running through the entire intestine; that Engelmann produced in dead animals "*Rollbewegungen*" by mechanical stimulation. Van Braam Houkgeest produced true *Rollbewegungen* in living animals by stimulating the peripheral end of one vagus after both splanchnics were cut. Nothnagel has seen in the living animal *Rollbewegungen*, but they never traversed the entire small intestine, and they could not be produced at will. Bokai produced by the introduction of CO_2 , CH_4 , and H_2S into the lumen of the intestine of living animals violent peristaltic movements which he termed "*rollende Bewegungen*."

We should add here that the statement of van Braam Houkgeest concerning the production of true *Rollbewegung* in the living animal by stimulation of the peripheral end of the vagus was, as far as we know, never tested by any one; in fact, we did not come across any reference to that statement, nor to the statement that the post mortem *Rollbewegungen* depend upon the vagi being intact.

The true *Rollbewegungen* were not seen by many students of intestinal movements. Those who had seen them would not have failed to dwell upon them, as they present a striking phenomenon.

In the extensive studies of Bayliss and Starling¹ this form of intestinal movement was apparently not observed by them. Had they observed this mode of peristalsis, they would not have failed to comment upon it, since it illustrated, as we shall show later, their law of intestinal contraction in a striking way. Starling² says that "the post mortem vermicular contraction described by Engelmann in the rabbit is probably merely an exaggerated wave just described," meaning pendular movements. He states there further that Mall places this form of contraction in a class by itself which he terms "vermicular." Mall, however, applies the term vermicular to the normal peristalsis as well as to the "irregular rapid wave." The latter is not designated by Mall by any special name. The term Rollbewegung does not occur in Mall's paper in which the description of Engelmann of the effect of mechanical stimulation of the intestines serves as a basis for the analysis of this mode of intestinal movement. Mall considers the rapid irregular wave as a pathological phenomenon "to rid the intestine rapidly of irritating products of decomposition," having in mind the above recorded statements of Bokai.

We came across the phenomenon of Rollbewegungen in our studies of the effects of saline purgatives,³ ergot,⁴ and of magnesium salts.⁵ We have seen it occasionally occurring in the living animal in the very same striking manner as was described by van Braam Houkeest in animals dying from asphyxia. In a series of experiments especially devoted to that subject we have traced at first the conditions under which Rollbewegungen accidentally occurred, and then attempted to bring out the phenomenon at will. It is the object of this paper to give a brief account of the results we have thus obtained. However, before entering upon a detailed description of our experiments we shall dwell upon the general appearance of the phenomenon of Rollbewegung and its essential features, by means of which it may be distinguished from other forms of intestinal movements.

Rollbewegung, or peristaltic rush. — In moderately distended and

¹ BAYLISS and STARLING: *Journal of physiology*, 1899, xxiv, p. 99, and 1900-1901, xxvi, p. 125.

² STARLING: SCHAEFER'S *Textbook of physiology*, ii, p. 329.

³ AUER: *This journal*, 1906-1907, xvii, p. 15.

⁴ MELTZER and AUER: *Ibid.*, p. 143.

⁵ MELTZER and AUER: *Ibid.*, p. 313.

moderately active small intestines, suddenly, frequently without warning, a rushing wave appears, which sweeps with great rapidity over the entire small intestine to stop only at the cæcum. Each coil, as the rushing wave passes through it, gives the appearance of a rapidly turning wheel. On account of twisted and intricate relations of the intestinal convolutions which do not permit the simultaneous observation of the entire gut, the peristaltic rush presents a confusing spectacle of whirling coils appearing, disappearing, and reappearing, until the entire rush comes to a standstill. The entire rush is at times accomplished in less than fifteen seconds. In the wave, as it hurries through each coil, two parts may be distinguished which present completely different aspects. In the aboral part of the wave the moving intestine appears in the shape of a turning wheel, is greatly distended, perfectly smooth, and offers apparently not the slightest resistance to the onward movement of its contents which is rapidly driven through it, and which consists of a dark brown or yellowish fluid intermingled with some gas bubbles. This part is apparently completely relaxed, all tonic or rhythmic contractions are inhibited. Closely at the foot of this section, at the oral end of it, the other part of the wave follows in which the intestine is contracted to a cord. The lumen of that part of the intestine is completely obliterated, but the constriction fails to produce such complete anemia as often attends strong contractions of the intestines caused by artificial stimulations, by the administration of barium, etc. Even at the height of the contraction the color is still pinkish. The contraction lasts only a few seconds, after which that part of the intestine looks patulous and empty; it retains, however, the rounded shape for some time. On account of the very rapid propagation of the wave of constriction the contracted piece of intestine appears sometimes to have a length of 5 to 10 cm. The preceding wave of inhibition extends apparently over a considerable section of the intestine; but its aboral end is usually lost in a hidden loop and cannot be ascertained.

A complete peristaltic rush begins somewhere in the duodenum and terminates at the cæcum. The exact starting-point of the wave is difficult to establish on account of the deep location of the duodenum.

Such complete Rollbewegungen are sometimes followed immediately by an incomplete wave which begins at some place in the

jejunum or ileum, and runs either a full course terminating at the cæcum or dies out, terminating at some distance from it.

As a rule, after such rushing waves, the small intestine remains completely quiescent, and for some time neither normal peristalsis nor pendular movements make their appearance.

Accordingly, the true and complete Rollbewegungen, as we have seen them, are characterized (1) by the great rapidity of progress of the circular constriction; (2) by the extensive and complete inhibition preceding the contraction, and (3) by the complete course of the wave, which traverses without interruption the entire small intestine.

In many instances, however, Rollbewegungen appear which are incomplete in one or the other of the characteristics mentioned. In the first place many rushing waves appear which run only short distances, beginning at the duodenum and terminating in some part of the jejunum or ileum, or beginning in a still lower section and dying out before reaching the cæcum. It is this kind of Rollbewegungen which were seen by Nothnagel in the living animal. In the second place, the progress of the wave may sometimes be of only moderate rapidity. Such slower waves may exceptionally traverse the entire intestine; as a rule, such slow waves die out in the middle of the circuit. However, even these slower waves move incomparably more rapidly than the waves of normal peristalsis. Finally, in some cases of Rollbewegungen the constriction as well as the relaxation may not be so extreme as described above; but here again both features of the rushing wave are even in such incomplete cases much more pronounced than in normal peristalsis.

Incomplete forms of Rollbewegungen are easily distinguishable from normal peristalsis by the size of the section which is involved, by the rapidity of the progress of the wave, and by the intensity of the processes, especially by the striking relaxation of the part of the intestine in front of the progressing constriction.

For those who have seen the phenomenon of Rollbewegungen it is hardly necessary to point out the particulars which distinguish them from pendular movements. The two types of movement can hardly be confounded, as they have practically very little in common.

It is, however, necessary to state expressly that the Rollbewegungen should not be confused with simple, violent constrictions of the small intestines, such as are frequently seen after intravenous

administration of barium chloride, of eserine, or even of ergot, and in many other conditions. These constrictions may even be stronger than those seen in Rollbewegungen, and may extend over 6 or 8 cm.; they might even show some travelling. But it is not difficult to distinguish them from the Rollbewegung. The constrictions in violent intestinal movements last a good deal longer than those of the Rollbewegungen, occur simultaneously at many sections of the small intestine; they do not travel progressively in an aboral direction, but move irregularly to and fro in a slow fashion. The striking differential point, however, is the absence in these violent constrictions of any inhibition in front of a constricted section. The contents of the intestine which is driven out by such violent constrictions have to pass through a more or less tonically contracted piece of intestine at the other end of which the way is frequently blocked by another strongly contracted part of the intestine.

We may say here that the above-mentioned "rollende Bewegungen," observed by Bokai after the introduction of CO_2 and other gases into the lumen of the intestines, were simply violent movements of the type just mentioned, and do not belong to the true Rollbewegungen. Bokai himself states positively that the inhibitory factor of the intestines was not involved in the action of these gases upon the intestines. The gases introduced into the lumen of the intestines stimulate the gut by contact to violent constriction, extending over a few centimetres, which constriction may extend farther down if the gases travel downward.

A few words more with reference to the nomenclature of this phenomenon. As stated above, the term Rollbewegungen was used by van Braam Houckgeest in his protocols. For lack of a better term he retained it also in the final publication of his studies. However, Rollbewegung indicates only the incidental feature of the phenomenon, the rapidity of the wave through the coils calling forth the illusion of a turning wheel. In the English literature there is no special name for this phenomenon. Cannon and other writers use the German term Rollbewegungen. As a fitting English designation for this phenomenon we adopted the term Peristaltic Rush, indicating in the first place its main feature, namely, the rushing character of the forward movement. Furthermore, "peristáltic" conveys the important fact that these movements possess the essen-

tial characteristic of peristalsis, namely, a contraction above and inhibition below (Law of Intestine of Bayliss and Starling). When the rushing wave runs through the entire small gut, we designate it as *complete*; when the wave runs through only a part of the course, or is deficient in other ways, we designate it as *incomplete*.

EXPERIMENTAL OBSERVATIONS.

Method. — The observations were made exclusively on rabbits, some of which received subcutaneous injections of morphin. The intestines were invariably observed in a warm saline bath. The saline consisted of a solution of 0.92 per cent sodium chloride, which is considered isotonic with the serum of the rabbit. (The solutions employed by van Braam Houkgeest consisted of 0.6 per cent sodium chloride.) In the present series of experiments the "receptacle for the bath" was prepared by flaps from the abdominal skin in the manner described in our paper on the action of ergot.¹ After an incision in the middle line of the abdomen the skin was extensively dissected on both sides from the underlying musculature, and by an appropriate suspension a deep receptacle was formed. This was filled up with a warm saline solution, and under cover of this the abdomen was freely opened in the linea alba. By slightly retracting the muscular walls by light weights the escaping intestines were bathed on all sides with the warm saline. The solutions were kept warm and at the proper level by frequent addition of fresh warm saline.

Movements in normal rabbits. — At the outset we may state that in normal living animals we have never seen movements of the small intestines which we could designate as rushing peristalsis according to our definition. In the present series the behavior of the intestines was watched for some time before the effect of any substance was tested. It must be admitted that the time given to such preliminary observations was necessarily not very long. But we have had sufficient opportunities in various series of experiments, carried out for other purposes, to watch the behavior of the uninfluenced intestines. Never have we noticed intestinal movements belonging to the rushing type.

After intestinal stimulants. — Neither have we seen rushing peri-

¹ MELTZER and AUER: This journal, 1906-1907, xvii, p. 143.

stalsis in experiments in which the intestines were stimulated to greater activity by subcutaneous or intravenous injections of some purgatives. In the experiments of one of us (A.) with subcutaneous and intravenous injections of sodium sulphate, sodium phosphate, and sodium citrate in which it was found, in agreement with the statement of J. B. MacCallum,¹ that the movements of the small intestine are increased, no peristaltic rush ever occurred, although the intestines were watched for hours. In experiments with intravenous injections of barium chloride and of eserine, in which the intestines were stimulated to violent and extensive contractions, no peristaltic movement was ever observed which bore the criteria of peristaltic rush as set forth above. The same we may state with regard to the effects of ergot, when employed alone. The intestines were stimulated to rhythmic, travelling, and tonic contractions, but none of them possessed the characteristics of rushing peristalsis; especially was the marked inhibitory wave absent from all the motor phenomena produced or aggravated by the injections of ergot or any of the other intestinal stimulants.

Stimulating and inhibitory factors.—The phenomenon of peristaltic rush we have seen to occur in such experiments in which apparently two opposing elements were in operation, — factors which increase intestinal activity and factors which as a rule inhibit this activity. Of the elements of the first class there were employed sodium phosphate, sodium sulphate, sodium citrate (“saline purgatives”), ergot, barium chloride, eserine, and destruction of some part of the dorsal cord. As inhibitory agents we have employed: calcium chloride, magnesium chloride, and magnesium sulphate. The greatest number of experiments were made with ergot and calcium, which gave, as we shall see later, very reliable results. We have obtained, however, satisfactory results also in other combinations. We shall illustrate our results only by a few greatly abbreviated protocols.

“Saline purgatives” and calcium. —

Experiment 1. — Gray female rabbit, 1750 gm. . . .

3.27 P. M. Abdomen opened in saline bath. . . . No movements of small intestines.

3.37 P. M. Injected subcutaneously 15 c.c. sodium phosphate (4 per cent). . . .

¹ J. B. MACCALLUM: This journal, 1904, x, p. 107.

3.47 P. M. Slight contraction of duodenum, balance of small gut empty and quiet. . . .

3.52 P. M. Duodenum full of light yellow fluid, quite active ; jejunum and ileum, still empty, show swaying movements. . . .

During next eighty minutes only slight changes.

5.10 P. M. Upper part of jejunum full, but not distended, shows constantly swaying movements. Duodenum shows only moderate swaying. . . .

5.17 P. M. Injected through the external jugular vein 2 c.c. sodium sulphate $m/8$ solution, followed by an injection of 1 c.c. saline. Small intestines show soon after a moderate increase of swaying motions.

5.23 P. M. Movements definitely less again.

5.30 P. M. Injected 2 c.c. sodium citrate $m/8$ solution, followed by 1 c.c. saline. Soon after the injection the movement of small intestines definitely increased, swaying and shortening movements, no constriction waves seen.

5.35 P. M. Intravenous injection of 2 c.c. CaCl_2 $m/8$ solution, followed by 1 c.c. saline.

Before injection was finished jejunum and ileum became quiet, but a part of the duodenum showed very active pendular movements and strong circular constrictions such as were not seen before.

5.40 P. M. Small intestines show again good swaying movements. . . .

5.50 P. M. Intravenous injection of 3 c.c. CaCl_2 $m/8$, followed by 1 c.c. saline. . . . No marked change in either direction.

6.00 P. M. Intravenous injection of 1 c.c. CaCl_2 $m/1$ solution, followed by 1 c.c. saline. Shortly after, a powerful contraction of the duodenum set in, shooting the fluid contents swiftly through the coils of the small intestines and stopping at the cæcum — a *complete peristaltic rush*.

Repeated twice with 1 c.c. CaCl_2 $m/1$, each time with the same result.

The above experiment, the protocol of which was here greatly abbreviated, is instructive in many directions. For two hours the intestines were watched while they were under the influence of subcutaneous and intravenous injection of the "purgative salts." As a result of these injections the small intestines showed a moderate increase of their motility which was confined to the swaying motions. At no time was there an indication of a rushing peristalsis. After the injection of 2 c.c. CaCl_2 $m/8$, which was equal to the preceding dose of sodium citrate, the activity of the jejunum and ileum became inhibited, which is in harmony with the statement of J. B. MacCallum that calcium counteracts the stimulating effect of the purgative salts.

¹ MACCALLUM, J. B.: This journal, 1904 x, p. 107.

The duodenum, however, became more active. After a few minutes the activity of the small intestines returned, and a second injection of a similar dose of CaCl_2 had no decided effect. Finally, when a dose of 1 c.c. of a *molecular* solution of calcium chloride was administered, which had to be injected very slowly, a powerful wave of rushing peristalsis swept over the entire small intestine. Repeating the injections with similar doses at proper intervals brought out similar results.

While we can confirm in general the discovery of MacCallum regarding the inhibitory effect of the calcium salts upon intestinal movements, we found at the same time that it is just the addition of the calcium salts which brings out the rushing peristalsis.

Similar results were obtained in some other experiments in which injections of calcium salts followed those of purgative salts.

However, we have not made many experiments with the purgative salts. Our main experiments were made, as stated before, with ergot and calcium, and we shall quote two abbreviated protocols to illustrate the various results obtained with this combination.

Calcium and ergot. —

Experiment 2. — Black female rabbit, 2030 gm. . . . Abdomen opened.
. . . Recovering from ether.

11.15 A. M. No movements of gut. Intravenous injection of 1 c.c. CaCl_2 *m/1*, followed by 2 c.c. saline.

11.23 A. M. Intestines relaxed, flat, no moments anywhere. . . .

11.35 A. M. Intravenous injection of 1 c.c. CaCl_2 *m/1*, followed by 2 c.c. saline.

11.42 A. M. Lower small gut shows slight motions, coils flat. . . .

11.54 A. M. Occasional good swaying of some loops of the small intestine.

11.55 A. M. Intravenous injection of 1 c.c. of fluid-extract of ergot (Squibb), followed by 2 c.c. saline. Shortly after, movements of small gut definitely increased. . . .

11.58 A. M. Strong contraction, a few centimetres long, drives contents swiftly into cæcum — *complete peristaltic rush*.

12.11 P. M. Intravenous injection of 1 c.c. ergot, followed by 2 c.c. saline.

12.13 P. M. Slight increase of swaying of small gut.

12.24 P. M. Swaying becomes once in a while more marked, but no sign of Rollbewegung.

12.26 P. M. Intravenous injection of 1 c.c. CaCl_2 *m/1*, followed by 2 c.c. saline.

12.27 P. M. Strong "Rollbewegung," driving contents into cæcum (stronger than before).

12.30 P. M. Small gut relaxed, but not empty.

12.40 P. M. Intravenous injection of 1 c.c. ergot, followed by 2 c.c. saline.

12.50 P. M. Pendular movements increasing, gut gradually filling up, no Rollbewegung.

12.52 P. M. 1 c.c. CaCl_2 $m/1$, followed by 2 c.c. saline. Swaying movements subsided for a while and then started again.

12.55 P. M. A good *wave of rushing peristalsis*. . . .

Animal killed by asphyxia. After convulsions subsided "good travelling peristalsis of small gut."

In this experiment the first injections of CaCl_2 in molecular solution produced no effect, there were previously no movements to be inhibited; the additional injection of 1 c.c. of ergot brought on within three minutes a wave of rushing peristalsis. A further injection of ergot brought out only swaying movements, but now an injection of CaCl_2 brought out within two minutes strong Rollbewegungen. Further injections of 1 c.c. ergot and 1 c.c. calcium brought on another peristaltic rush.

Calcium chloride alone had no effect at all (when there were no previous movements), and ergot alone brought only an aggravation of the usual intestinal movements; but when ergot followed calcium and when calcium followed ergot, the result was usually a complete peristaltic rush.

Experiment 3. — Female rabbit, 1530 gm. . . .

11.35 A. M. Abdomen open, all operations finished. No movements of intestines visible.

11.50 A. M. Occasional slight movements of small intestine.

11.54 A. M. Intravenous injection of 1 c.c. CaCl_2 $m/1$, followed by 2 c.c. saline.

11.57 A. M. Moderate, but distinct, pendular movements of all visible coils of small gut.

12.01 P. M. Movements improved; some coils filled up, are round; duodenum invisible.

12.11 P. M. Intravenous injection of 1 c.c. CaCl_2 $m/1$, followed by 2 c.c. saline. Before injection of the calcium was finished all movements disappeared.

12 15 P. M. Slight movements visible again.

12.20 P. M. Movements improved, present in nearly all visible coils.

12.28 P. M. Intravenous injection of 1 c.c. of ergot, followed by 2 c.c. saline. Before the injection of saline is finished a *moderate incomplete peristaltic rush appeared*.

12.32 P. M. Pendular movements in all coils.

12.34 P. M. Nearly all coils are perfectly quiet.

12.44 P. M. Intravenous injection of 1 c.c. of ergot, followed by 2 c.c. saline.

12.46 P. M. Pendular movements in all coils, gradually increasing.

12.58 P. M. No Rollbewegung occurred. Intravenous injection of CaCl_2 *m/1* was given again, followed by 2 c.c. saline. Before injection of calcium was finished, *an incomplete but strong peristaltic rush set in*.

1.02 P. M. All coils very active.

1.12 P. M. *Complete peristaltic rush*.

1.27 P. M. Slight pendular movements in some coils.

1.28 P. M. Intravenous injection of 1 c.c. of ergot, followed by 2 c.c. saline.

1.30 P. M. Pendular movements increased everywhere.

1.38 and 1.44 P. M. *Incomplete but good waves of rushing peristalsis* reach the cæcum. Loops remain full, round, and show good swaying movements.

1.45 P. M. Intravenous injection of 1 c.c. of CaCl_2 *m/1*, followed by 2 c.c. saline.

1.47 P. M. Entire gut quiet.

A few more alternating injections of calcium and ergot brought out only increased normal activities and their inhibition, but no waves of rushing peristalsis.

In this experiment the various alternating injections of calcium and ergot brought out only one complete peristaltic rush and a few incomplete rushes.

In many of the experiments upon normal animals, in which ergot and calcium were used, more than one complete peristaltic rush occurred, besides a few incomplete waves. Frequently, however, after one or two rushes occurred, further injections of these substances became less effective and an incomplete Rollbewegung was the most that could be obtained. We had no experiment in which the injection of calcium and ergot failed completely to cause rushing peristalsis, and we had only one experiment in which only incomplete Rollbewegung occurred. In this experiment morphine alone was used as an anesthetic, the respiration became very slow, and ergot failed otherwise to cause the customary stimulating effect upon the intestinal movements.

Destruction of cord and administration of calcium.—In the following instructive experiment the administration of calcium alone brought out a complete peristaltic rush.

Experiment 4.—White female rabbit, 1560 gm. Morphine subcutaneous 0.015. . . . Spinal cord destroyed below fifth dorsal vertebra. . . . Abdomen opened.

11.45 A. M. Good swaying motions and constrictions all over the small gut.

12.00 M. Intravenous injection of 1 c.c. of CaCl_2 m/1. Before injection was finished, a strong wave swept over entire small intestine (duodenum not visible) and drove contents into cæcum. *Complete peristaltic rush.* After this wave passed, gut became quiet.

12.06 P. M. Another wave swept down, stronger than before. Small gut, after wave, empty and tape-like, moderately relaxed and shows some swaying.

Three more injections of CaCl_2 did not bring out any rushing peristalsis.

Here the first injection of calcium brought out two waves of rushing peristalsis. The destruction of the cord, which usually greatly increases the activity of the intestines, supplied the stimulating factor; the intestines were very active while the calcium injection was given.

Magnesium salts and ergot.—Rushing peristalsis was rarely brought about when in addition to ergot (and destruction of the cord) magnesium sulphate or chloride was injected instead of calcium. Out of five experiments only in one two incomplete Rollbewegungen occurred. As we have shown elsewhere,¹ magnesium salts inhibit completely the movements of the intestines produced by the injection of ergot. In the present line of experiments the effect of ergot is apparently nearly completely lost as a stimulating factor in the presence of the strongly inhibitory effect of magnesium.

Calcium chloride and barium.—On the other hand, the inhibitory effect of calcium is apparently a less reliable factor for the production of peristaltic rush in the presence of such strongly stimulating substances as BaCl_2 . In five experiments in which calcium chloride and barium chloride were injected intravenously, in three no peri-

¹ MELTZER and AUER: This journal, 1906-1907, xvii, p. 318.

stalsis of a rushing type made its appearance. In the two other experiments one complete peristaltic rush occurred in each one, besides one or two incomplete waves.

We are here reminded of the statement of MacCallum¹ that "the peristaltic movements produced by barium chloride are usually not stopped by the administration of calcium."

Magnesium and barium. — Of four experiments in which barium and magnesium chloride were alternately injected, in three there were a few complete peristaltic rushes as well as incomplete waves, and only in one all signs of *Rollbewegungen* were missed. In our previous experiments² we reported that magnesium salts are capable of inhibiting the violent intestinal constrictions produced by barium. While the inhibiting effect of magnesium is apparently much greater than that of calcium, and is strong enough to overpower temporarily the violent constrictions produced by barium, the stimulating effect of the latter is, however, too strong to be completely annihilated, even by magnesium. The result of the alternating injection of the two strong antagonistic factors is therefore often a compromise in the shape of a peristaltic rush.

In the following experiment calcium as well as magnesium was employed:

Experiment 5. — Gray female rabbit, 1320 gm. Morphine 0.01. . . . Abdomen opened at 11.50.

11.55 A. M. No sign of motion anywhere. Intravenous injection of 8 c.c. CaCl_2 *m/8* followed by 2 c.c. saline.

12.00 M. No effect.

12.05 P. M. Injection of 0.5 c.c. BaCl_2 *m/8*. Strong contractions of small gut, no "running."

12.15 P. M. 8 c.c. CaCl_2 *m/8*, later 0.5 c.c. BaCl_2 *m/8*.

12.20 P. M. Again 0.3 c.c. BaCl_2 *m/8*. "No runs or anything approaching them."

12.30 P. M. 4 c.c. CaCl_2 *m/8* and 2 c.c. saline. Small gut quieter at first, then attempt at running, but no definite wave of contraction swept along.

12.50 P. M. 4 c.c. CaCl_2 *m/8* and 2 c.c. saline. Soon after 0.1 c.c. BaCl_2 *m/8*. Same as before: tonic contractions of some parts of small gut.

1.40 P. M. 0.9 c.c. MgCl_2 *m/1* and 0.1 BaCl_2 *m/1*. Shortly after a definite run occurred over entire small gut. Later two short runs occurred.

¹ MACCALLUM: This journal, 1904, x, p. 107.

² MELTZER and AUER: *Ibid.*, 1906-1907, xvii, p. 318.

The several alternating injections of calcium and barium brought no success, while the first injection of $MgCl_2$ brought on a true Rollbewegung.

Eserin with calcium or magnesium. — We also made a few experiments with eserin. In the experiments in which calcium alone was used with eserin, there was practically no success. In one experiment, however, in which at first eserin was alternated with $MgSO_4$ no running waves occurred. Later, however, when calcium was substituted for magnesium, the injections of calcium at first quieted the contractions, but a few minutes after each injection a definite complete peristaltic rush set in.

Destruction of cord. — The cord was destroyed in seven experiments (below the 5th, 3d, or 2d dorsal vertebra). In four experiments the abdomen was opened soon after the destruction and watched only for fifteen to twenty minutes before an injection of any kind was given. The intestinal activity was increased in all four experiments, but no rushing peristalsis was seen. In three experiments the abdomen was opened about two hours after the destruction of the cord. In two of these experiments there were a few spontaneous peristaltic rushes, complete and incomplete. In the third experiment there was only one incomplete peristaltic rush, but the intestines in this experiment have also otherwise shown very little activity.

From these few experiments we learn at least in a general way that the phenomenon of peristaltic rush may occur in rabbits whose cord was so destroyed as to eliminate the influence of the splanchnics and who otherwise did not receive any substance capable of inhibiting intestinal movements.

Section of vagi. — According to van Braam Houkgeest, as will be remembered, the post mortem Rollbewegungen did not occur when both vagi were previously cut. We have made four experiments in which $CaCl_2$ and ergot were given alternately after both vagi were cut. In none of these cases did a complete Rollbewegung occur. In three experiments there were some incomplete Rollbewegungen, in two of which the course of these waves was short and sluggish and marked by a very slow relaxation of the contracted part. In one of these experiments the vagi were cut after the animal had already received a few doses of ergot and calcium, but only one good but incomplete Rollbewegung was produced. After cutting the vagi and continuation of the injections, one other incomplete

Rollbewegung occurred which was as good as the one before cutting the vagi. In this case after killing the animal by asphyxia an incomplete Rollbewegung occurred exactly like those observed while the animal was alive.

These few observations seem indeed to justify the assumption that the occurrence of a true complete peristaltic rush is to a great measure dependent upon the integrity of the vagi.

We may call to mind here that cutting the vagi interferes greatly, for some time at least, with the normal movements of the stomach and also, as we have shown recently,¹ with the normal movements of the rabbit's cæcum.

Stimulation of the vagi.— Van Braam Houkgeest has also stated that stimulation of the peripheral end of one vagus will also cause in the living rabbit Rollbewegungen, provided both splanchnics are previously cut. We have tested this claim in two rabbits whose splanchnics were cut, and in three others in which the cord was destroyed, which was equivalent to cutting the splanchnics. In no case could we find that stimulation of the vagi brings out complete or incomplete peristaltic rushes. Even in an experiment in which, after destruction of the cord, rushing peristalsis occurred spontaneously and with readiness, stimulations of the peripheral end of the vagi, although visibly aggravating the other motor activities of the intestines, did not contribute to the production of a peristaltic rush.

Our experiments have shown that the phenomenon of peristaltic rush occurs when the animal receives injections of two groups of substances. The first group comprises sodium sulphate, sodium phosphate, sodium citrate, ergot, barium chloride, and eserine. The second group comprises calcium chloride, magnesium chloride, or magnesium sulphate. The substances of the first group are intestinal stimulants; that is, by their injection the intestines are stimulated to greater activity. The second group we consider as inhibitory substances for the intestinal movements; that is, we assume that by their injection intestinal movements, when present, are reduced or completely inhibited for some time. The stimulating character of the first group of substances is well understood and requires little discussion. Among this group the sodium salts are the weakest stimulants, ergot acts much more strongly, and barium

¹ Proceedings of the Society for Experimental Biology and Medicine, 1907, iv, p. 37.

and eserine have the strongest effect. With regard to the inhibitory group a few explanatory remarks would not be out of place. The inhibitory effect of calcium upon the intestinal movements was observed by J. B. MacCallum. These movements, which were started or aggravated by the injection of "purgative salts" were inhibited by the injection of calcium chloride. This discovery was made on the basis of J. Loeb's well-known view of the general inhibitory effect of calcium salts. The strong effect of barium chloride could not be inhibited by the injection of calcium. From the observations in the present series of experiments as well as on many other occasions, we can confirm the statement that the injection of calcium chloride causes as a rule an inhibition of intestinal movements when normally present or brought on by the injection of stimulating agents.

Regarding the inhibitory effect of magnesium salts upon intestinal peristalsis, we have dealt recently in a special article on that subject.¹ We have found that these salts are capable of inhibiting intestinal movements of whatever source. We may state again expressly that the violent movements of the intestines caused by barium or eserine can also be inhibited by the injection of magnesium salts.

We may mention here that MacCallum also observed that intravenous injection of magnesium chloride inhibits the movements of the intestines produced by sodium citrate, sulphate, etc., although he surprisingly stated that subcutaneous injection of magnesium sulphate has a stimulating effect upon the intestines. In our experience we found no difference between magnesium sulphate and magnesium chloride; both inhibit the intestinal movements.

We may also state expressly that according to our experience the inhibition exerted by magnesium salts is distinctly stronger than that produced by calcium; the contractions are more completely inhibited, the effect lasts longer, and we met with no kind of movements which could not be reduced or abolished by magnesium, while calcium, according to MacCallum, cannot overcome the effect of barium chloride.

As to the meaning of inhibition we may refer to our first paper on the magnesium salts.² We started from the hypothesis that magnesium favors such an action as that of the vagus nerve. For

¹ MELTZER and AUER: This journal, 1906-1907, xvii, p. 313.

² This journal, *loc. cit.*

the intestines we may say that the effect of magnesium is similar to the well-known inhibitory action of the splanchnics.¹

Are our present results in harmony with this hypothesis? Did we not find that by the injection of calcium or even magnesium a most remarkable intestinal movement takes place? We must admit that any investigator who would come across the phenomenon of peristaltic rush occurring after an injection of calcium or magnesium chloride without having much experience with these salts, might be inclined indeed to insist that calcium and magnesium are stimulating agents for intestinal movements.²

We shall, however, call attention to the following facts. If injections of magnesium or of calcium are given in large or small doses to an animal which previously received no stimulating salts and whose cord was not destroyed or whose splanchnics were not cut, no movement of the intestines ever follows those injections. For magnesium we may state that this is the absolute rule, to which apparently there is no exception. For calcium there is once in a while an exception; we have occasionally seen after an injection of calcium chloride a constriction appearing in some part of the gut, but this was a rare occurrence, and the effect was circumscribed and very brief. The general rule is that calcium produces no intestinal contractions. Furthermore, if there have been slight spontaneous movements of the intestines, or slight movements brought on by the injection of some intestinal stimulants, an injection of magnesium or calcium will invariably stop these movements for a shorter or longer period. Finally, even when the intestines show strong activity, "spontaneous" as well as those brought on by artificial means, in the great majority of the cases an injection of

¹ See also A. G. MAYER: Rhythmical pulsation in *Scyphomedusa*, Carnegie Institution publications, 1906.

² The situation with which Loeb was confronted in his studies upon the hydromedusa *Polyorchis* (The stimulating and inhibitory effects of magnesium, etc., *Journal of biological chemistry*, 1905-1906, i, p. 427) is of a similar misleading character. When to a solution of NaCl in which the medusa does not show any movements, magnesium chloride is added, the characteristic swimming movements soon appear. This conveys the impression that magnesium acts as a stimulating agent. The probable interpretation, however, is that the sodium chloride solution keeps the muscle in a state of contraction, a systolic state, and that the addition of magnesium causes a relaxation of the tonus, thereby favoring the reappearance of rhythmic diastoles. In favor of that view LOEB mentions the fact that the mouth and tentacles are permanently contracted in any solutions without magnesium.

magnesium or calcium will cause at least a preliminary inhibition of the intestinal movements.

We therefore assume that the injection of magnesium or calcium introduces an inhibitory factor, and that the appearance of the phenomenon of peristaltic rush occurs only as a compromise between two opposing factors, the stimulating and inhibitory elements. When we stated above that we were enabled to produce at will the occurrence of peristaltic rush in living rabbits, we did not mean to claim that certain injections will invariably bring out the phenomenon. We claim only that we are now in a position to create a situation in which the phenomenon in all probability is likely to occur. The peristaltic rush by no means promptly follows each injection. On the contrary, in a prolonged experiment in which many alternating injections were given, it frequently happened that only one or two complete waves of rushing peristalsis made their appearance.

The phenomenon of peristaltic rush consists, as we have analyzed above, of two parts, — of a stimulating part, in which the circular constriction is strong and its propagation rapid, and of an inhibiting part, in which a long section of the intestine in front of the rushing wave of constriction is completely relaxed, so as to offer no obstacle to the swiftly driven contents. The peristaltic rush is in its composition very similar to the normal peristalsis of the œsophagus in which an inhibitory wave runs rapidly ahead of the contraction to clear the path of all obstructing constrictions. By introducing at the same time into the body stimulating and inhibiting agents, conditions are created which permit the occurrence of such specific combinations of stimulation and inhibition as to start off the wave of peristaltic rush.

The antagonistic factors, to be favorably combined, must be mated in proper proportions. The stimulating effect of ergot, which is not very strong, is best combined with calcium, the inhibitory effect of which is also not too strong. The inhibitory effect of magnesium is too strong and is apt to completely overpower the stimulation of ergot. On the other hand, the strong stimulating effect of barium is better paired with magnesium than with calcium in order to bring out peristaltic rush.

The increased activity of the intestines after release from the inhibitory grip of the splanchnics (*i. e.*, after their section) is apparently just of the right proportion to enter into a satisfactory com-

bination with the inhibitory effect of calcium. Hence the occurrence of peristaltic rush after an injection of calcium when the splanchnics were previously cut.

The occurrence of peristaltic rush after destruction of the dorsal cord would seem to require some explanation. The character of the compromise is here not very evident, since the destruction of the cord rather removes an inhibitory factor. * More facts will have to be collected before we could discuss this point satisfactorily. But we may say that in our experiments on the rabbit's cæcum¹ we have established the fact that simple opening of the abdomen even in a warm saline bath is an inhibitory stimulus of a local character for the cæcum. It is probably an inhibitory stimulus also for the small intestine.

Finally, we have to recall here our observation according to which the vagi are apparently controlling factors in the management of peristaltic rush. In the absence of their influence no complete peristaltic rush takes place. Incomplete, short, sluggish runs do occur even after the vagi are cut, and they may be of peripheral origin, either myogenic or neurogenic. But a complete true peristaltic rush seems to require the assistance of the central nervous system conveyed through the vagi.

Peristaltic rush is probably not an infrequent occurrence in various pathological conditions, and is probably also an essential factor in purgation. As to normal conditions, we have stated above that we have never seen Rollbewegungen in the opened abdomen of a normal animal. But an opened abdomen is not a normal state. We have shown that the clearly visible movements of the stomach² and of the cæcum³ completely disappear after opening of the abdomen. We will, however, not enter here into a discussion of that subject.

RÉSUMÉ.

Peristaltic rush (Rollbewegungen) consists of a rapidly progressing wave of contraction preceded by a completely relaxed long section of the intestine through which fluid contents mixed with gas bubbles is rapidly driven. A complete peristaltic rush is one

¹ MELTZER and AUER: Zentralblatt für Physiologie, 1907, xxi, p. 71:

² AUER: This journal, 1906-1907, xvii, p. 15.

³ MELTZER and AUER: Proceedings of the Society of Experimental Biology and Medicine, 1907, iv, p. 37.

which sweeps down from the duodenum to the cæcum without stopping.

Peristaltic rush was seen to occur in living animals with opened abdomen when intravenous injections of stimulating and inhibitory substances were given. As stimulating substances were used some purgative salts, ergot, barium chloride, and eserin; and as inhibitory substances, calcium chloride, magnesium chloride, and magnesium sulphate were used. The best success was obtained by ergot and calcium chloride.

Cutting the vagi prevents the occurrence of complete peristaltic rush.

As a result of a general character we may consider the fact that the simultaneous administration of stimulating and inhibitory substances did not lead to a mutual neutralization, but rather to a condition in which both effects are manifest and are combined in such a co-ordination as to favor effective motion, namely, an increase of the motor factors and an inhibition of the antagonists, — a condition which was designated by one of us (M.) as *contrary innervation*.

**THE OPSONIC INDEX IN ERYSIPELAS AND ITS RELATION TO
TREATMENT BY INOCULATION OF KILLED
STREPTOCOCCI.**

BY EDWIN H. SCHORER, M.D.,
OF NEW YORK.

(From the Rockefeller Institute for Medical Research, New York.)

AFTER the observations of Wright and Douglas had stimulated investigations of all diseases of infectious origin, Dr. James C. Ayer kindly placed at our disposal for the determination of opsonic indices and for vaccination all cases of erysipelas treated in the wards of Bellevue Hospital, from January 1 to February 15 of this year.

During this time about 100 cases of erysipelas were admitted. Many of these cases were not suitable for investigation, as the patients entered the hospital at a late stage of the disease, after desquamation had occurred, or after there was complication with other diseases. Those cases in which no definite diagnosis of erysipelas could be made out were not studied. Altogether, the opsonic index was studied in 36 patients, and 37 patients received one or more injections of killed cultures.

The etiology of erysipelas was well established by the work of Fehleisen, who regarded *Streptococcus erysipelatos* as the cause of the disease. It is probable that streptococci causing erysipelas do not differ from those causing wound infections, for erysipelas often follows wound infections—infections of the mouth and scarlet fever. Moreover, streptococci from erysipelas transferred to other individuals may cause puerperal fever and other infections. In our series, erysipelas frequently followed coryza and wound infections.

Streptococci were isolated from cases of cellulitis and from blebs upon the skin in cases of erysipelas. Although it is possible that infection with streptococci can be best treated with killed cultures isolated from the case in question, the same culture being used for the determination of the opsonic index, yet the course of the average case of erysipelas makes it impracticable to isolate cultures from every case of the disease and to make a vaccine for its treatment. In many cases no blebs are formed, and even if a bleb were artificially produced, the patient might have recovered before the

culture could have been isolated and the vaccine made. Moreover, since most patients are admitted to the hospital during the acute or during the advanced stage of the disease, treatment to be of service must be administered without delay.

A uniform mixture of streptococci from 4 cases of erysipelas was used in determining opsonic indices and in making vaccines. One of these cultures came from a bleb present with facial erysipelas and another was obtained from the discharging ear of a patient with erysipelas of the ear and otitis media; the third culture was from a case of erysipelas affecting the leg, and a fourth was from a bleb with erysipelas of the face and scalp. All of these microorganisms had the same cultural characteristics, and grew in long chains in bouillon. They produced hemolysis, caused acid formation in milk, and did not ferment inulin.

TECHNIQUE OF DETERMINING THE OPSONIC INDEX. One tube of each of the first two cultures and two tubes of each of the third and fourth cultures were incubated twenty-four hours on glycerin-glucose-agar, to which 20 per cent. of sterile sheep serum had been added. From 2 to 3 c.c. of sterile salt solution was used to prepare an emulsion from these six slant agar growths. The emulsion was put in a small test tube containing some sterile sea sand; the tube was sealed in the flame and kept during one and a half hours in the shaking machine. The sand and emulsion in the tube were centrifuged for one minute and the fluid was drawn off. In this way, short chains of from two to four cocci were uniformly obtained.

The serum used for control was a mixture from two healthy adults. Our leukocytic emulsion was obtained by drawing ten drops of blood into about 10 c.c. of 1.5 per cent. potassium citrate in normal salt solution. The mixture was centrifuged, and without further washing the leukocytes were drawn off.

The mixture of leukocytes, bacteria, and serum in capillary tubes was incubated in the opsonizer of Wright at 37° C. for twenty minutes. The slides were fixed with methyl alcohol and stained with Loeffler's methylene blue. The cocci in fifty definitely outlined and isolated polynuclear leukocytes along the upper and low borders of the spread were counted. The slides uniformly showed well-preserved leukocytes and the chains of cocci were well broken up.

This method of determining the opsonic index does not differ essentially from the one used by Wright and Douglas. A series of experiments was made to determine the accuracy of the method. In these tests, *Staphylococcus aureus*, of which suspensions are easily prepared, was used instead of *Streptococcus erysipelatis*. The attempt was made to determine (1) the limits of error when several specimens of serum are drawn almost simultaneously from the same individual, and (2) the variation of the opsonic power of healthy individuals.

I. Five specimens of the blood of a healthy adult were drawn from five fingers of the same hand. The leukocytes used for the determination were washed twice in 1.5 per cent. potassium citrate in normal salt solution.

TABLE I

Specimen	1st 100 leukocytes counted.	2d 100 leukocytes counted.	Phagocytic index.
1	166	196	1.810
" 2	176	183	1.795
" 3	198	199	1.985
" 4	185	175	1.800
" 5	230	190	2.100

II. A series of ten specimens of blood from ten healthy adults was studied to determine the variations in opsonic power in healthy individuals. The leukocytes were twice washed with 0.85 per cent. salt solution. The number of cocci taken up by one hundred polynuclear leukocytes was counted in two preparations separately incubated, and counted by two workers, A and B. The phagocytic indices are as follows:

TABLE II.

Source.	A	B
N.	9.48	7.63
J.	9.16	5.20
B.	8.75	6.80
McL.	7.27	4.37
Z.	6.63	5.12
C.	6.60	5.37
S.	5.89	1.82
O.	5.84	1.57
H.	5.49	6.03
Ns.	4.91	4.37

In the early observations of Wright and Douglas, the opsonic index was determined by comparing the serum to be tested with a mixture of several normal sera (pooled serum). This practice has been almost universally discarded, comparison being made with a single serum. The great error introduced by this method is shown by tables III and IV, which contain the indices obtained by using each serum for comparison with all of the others. Table III (Series A) shows that the normal opsonic index varies from 0.52 to 1.93, and in Table IV (Series B) it varies from 0.57 to 1.75.

TABLE III—A.

		Numerator.									
		I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.
Denominator.	I.	...	0.97	0.86	0.77	0.70	0.70	0.62	0.62	0.58	0.52
	II.	1.03	...	0.89	0.79	0.72	0.72	0.64	0.64	0.60	0.52
	III.	1.15	1.12	...	0.89	0.81	0.80	0.72	0.72	0.67	0.60
	IV.	1.30	1.26	1.12	...	0.91	0.90	0.81	0.80	0.76	0.68
	V.	1.42	1.38	1.23	1.10	...	1.00	0.89	0.88	0.83	0.74
	VI.	1.42	1.39	1.23	1.10	1.03	...	0.89	0.88	0.83	0.74
	VII.	1.61	1.56	1.38	1.23	1.13	1.12	...	0.99	0.93	0.83
	VIII.	1.62	1.57	1.39	1.24	1.13	1.13	1.01	...	0.91	0.84
	IX.	1.73	1.67	1.48	1.32	1.21	1.20	1.07	1.06	...	0.89
	X.	1.93	1.87	1.66	1.48	1.33	1.32	1.20	1.19	1.10	...

TABLE IV—B.

		Numerator.									
		I.	III.	IX.	VI.	II.	V.	VII.	VIII.	X.	IV.
Denominator.	I.	...	0.89	0.79	0.70	0.68	0.67	0.63	0.60	0.57	0.57
	III.	1.12	...	0.89	0.79	0.76	0.75	0.71	0.67	0.64	0.64
	IX.	1.27	1.13	...	0.89	0.86	0.85	0.80	0.76	0.72	0.72
	VI.	1.42	1.27	1.12	...	0.97	0.95	0.90	0.85	0.81	0.81
	II.	1.47	1.31	1.14	1.02	...	0.98	0.93	0.88	0.84	0.84
	V.	1.49	1.33	1.17	1.05	1.02	...	0.94	0.89	0.85	0.85
	VII.	1.59	1.41	1.25	1.11	1.08	1.06	...	0.95	0.91	0.91
	VIII.	1.67	1.49	1.21	1.13	1.14	1.12	1.05	...	0.96	0.96
	X.	1.75	1.56	1.38	1.23	1.19	1.17	1.10	1.05	...	1.00
	IV.	1.75	1.56	1.38	1.23	1.19	1.17	1.10	1.05	1.00	...

III. Blood was taken from five healthy individuals and a pool was made by mixing equal volumes of each of their sera. The phagocytic indices were determined in duplicate after separately incubating and counting the opsonic preparation, and an average of these determinations was compared with the phagocytic index obtained with the pooled serum. The phagocytic indices determined as the result of counting 100 polynuclear leukocytes in each preparation were as follows:

	Z.	N.	J.	McL.	S.	Pool.	Calculated average.
A	6.08	7.64	5.61	5.88	7.25	6.49	6.57
B	6.89	7.79	7.78	7.11	7.17	7.20	7.75

IV. This experiment was made with blood from five individuals, one of whom suffered with a streptococcic and staphylococcic infection, and another with a staphylococcic infection. A pool was made of the five sera and the phagocytic index determined by counting the cocci in 50 polynuclear leukocytes.

	B	C
1. Normal	2.72	3.004
2. Normal	2.24	3.160
3. Normal	3.06	3.960
4. Streptococcic and staphylococcic infections	2.08	3.100
5. Staphylococcus aureus infection	3.40	4.080
6. Pool of five sera	2.80	3.300
Calculated pool	2.70	3.460

It is evident that the method of determining the opsonic index usually employed is not accurate. There is not always agreement between results obtained by counting 100 and 200 polynuclear leukocytes on the same slide. When separate specimens of serum from the same individual are made, there is even greater error. When sera from different healthy individuals are tested by comparison with a single normal individual, such great error is introduced that the determination has little value. The preceding tests show, moreover, that comparison of the serum to be tested with a pooled serum decreases this range of error.

Observations upon the opsonic index were undertaken with the methods recommended by Wright and Douglas, and subsequently employed almost universally. The error introduced by comparison with a single serum was diminished by the use of a mixture of two normal sera. To further diminish the errors which occur with all determinations of the opsonic index, composite charts embodying a considerable number of observations have been prepared, when possible. The possible error introduced by collecting observations made upon a variety of cases, some of which were complicated by conditions other than erysipelas, is recognized.

VACCINE. Twenty-four-hour cultures of the same strains of streptococci used for testing the opsonic index were used to make the vaccine, which was standardized by estimating the number of cocci per cubic centimeter.

OPSONIC INDEX IN ERYSIPELAS. Before determining the changes in opsonic index produced by vaccination, a number of unvaccinated cases were studied. The most instructive of these cases are the two which follow.

CASE I.—S., a man, aged thirty-eight years, who had a migratory, recurrent erysipelas of the face, ears, scalp, and neck, was admitted to the hospital on the fourth day of his disease. When his face was involved, his index was 0.7; when it was desquamating, the index had risen to 1.3; when his face was again involved, seven days later, the index was 1.4; and when it was desquamating again, the index had dropped to 0.8. When his neck became involved,

one day after his face began to desquamate for the second time his index was 1.0; two days later, with an index of 1.1, his back became involved, and three days later, with an index of 3.2, his shoulders and neck were again involved, and two days later, with an index of 1.1, the back and shoulders began to desquamate. (Chart I.)

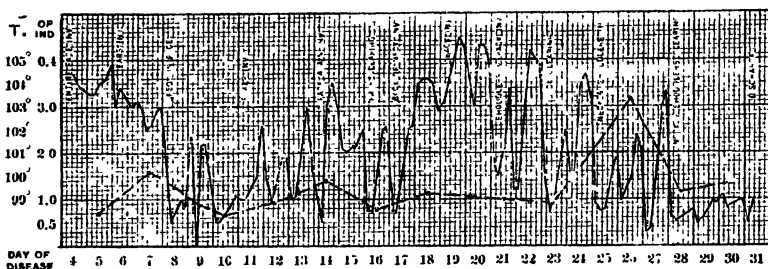


CHART I.—Temperature (unbroken line) and opsonic index (broken line) in a case of erysipelas (Case I).

CASE II.—A, a man, aged twenty-four years with erysipelas of the face and ears, was admitted on the eighth day of the disease with an index of 0.9. The next day his temperature dropped and desquamation began; his index was found to be 1.1. Subsequently the index, though the patient was perfectly well, remained below unity. (Chart II.)

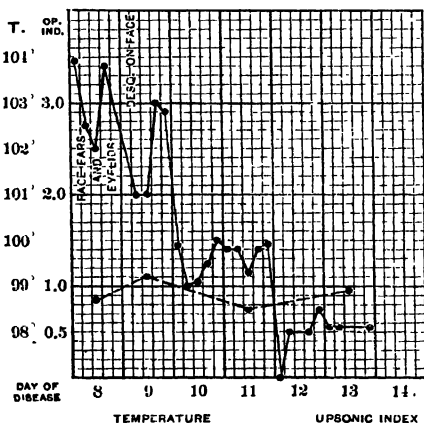


CHART II.—Temperature (unbroken line) and opsonic index (broken line) in a case of erysipelas (Case II).

In these two cases there has been no constant change of index corresponding with desquamation and recovery.

To further study the relation of the opsonic index to the course of the disease, the indices of all patients who had received no vaccine were tabulated (Table V) with regard to the day of the disease, and an average opsonic index for each day was determined.

TABLE V.

TABLE SHOWING THE OPSONIC INDEX AT THE TIME OF ADMISSION, BEFORE KILLED CULTURES WERE INJECTED, AND THE DAY OF THE DISEASE.

Day of disease.										
1	2	3	4	5	6	7	8	9	10	11
0.6	1.2	0.6	0.7	0.7	0.4	0.9	0.8	1.1	0.6	1.0
0.3	1.1	1.1	1.1	1.0		1.1	0.8	0.9		0.7
	1.0	2.4	1.4	0.9		0.6	1.3	1.7		1.0
		1.7	1.5	0.6		1.6				
			0.7	1.1						
			1.0							
			1.2							
0.45	1.1	1.45	1.1	0.86	0.4	1.05	0.96	1.23	0.6	0.9
Average.										

The composite chart indicates that erysipelas causes an increase of the opsonic index which reaches its maximum about the third day of the disease, and is followed by a gradual fall. The subsequent course of the chart represents, in very large part, observations made upon recurrent, migratory, and complicated cases. Ruediger¹ found that the index was high during an attack of erysipelas.

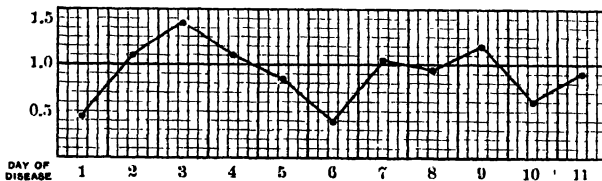


CHART III.—To show the average opsonic index and the day of disease before killed cultures of streptococci were injected.

RESULTS OF INJECTIONS OF KILLED CULTURES. During the investigation, 37 patients were injected with streptococci killed by heat. The number of cocci used for injection varied from 25,000,000 to 100,000,000. The effect on the opsonic index was studied in 10 patients receiving 25,000,000 cocci, in 5 patients receiving 50,000,000 cocci, and in 11 patients receiving 100,000,000 cocci. The results are indicated in the following tables with the opsonic index, before and after injection.

¹ Jour. Amer. Med. Assoc., 1906, xli, 108.

OPSONIC INDEX OF PATIENTS RECEIVING 25,000,000 STREPTOCOCCI.

	Before vac.	1 day after vac.	2	3	4	5	6	7	8	9	10
1	1.1	...	0.7	1.2	1.2	2.8			
2	1.0	3.0	2.1	1.7							
3	1.7	0.9	...	0.8				
4	0.6	1.5	...	0.7	4.1				
5	1.0	...	1.0	0.9	0.9		0.8	
6	0.7	...	1.3	...	0.9	1.1	...	1.0			
7	2.4	...	0.8	2.1	2.5	1.7					
8	1.2	...	1.3	1.0	...	1.1	1.7	1.1			
9	0.4	1.4	1.3	...	1.1	...	0.8		
10	0.3	1.1		1.7							
Aver.	1.04	1.83	1.2	1.48	1.4	1.16	1.78	1.45	0.8	0.8	

OPSONIC INDEX OF PATIENTS RECEIVING 50,000,000 COCCI.

1	1.0	0.8	...	0.8	0.7		
2	1.4	1.3	1.0	
3	0.8	1.4	1.2	...	0.8	...	1.2				
4	1.1	1.0	1.2	...	1.2	...	1.1	...	0.9		
5	0.6	0.4	...	1.0	...	1.1	...	1.0	0.9	...	1.1
Aver.	0.98	0.98	1.2	0.9	1.0	1.1	1.15	1.0	0.83	1.0	1.1

OPSONIC INDEX OF PATIENTS RECEIVING 100,000,000 COCCI.

1	1.2	0.5	...	0.5	...	1.3	0.5	0.6	0.8
2	1.0	1.0	...	0.9	...	0.8					
3	1.0	...	1.1	0.8	1.9				
4	1.1	0.3									
5	0.9	...	1.2	...	0.6	1.0	0.8				
6	1.0	0.8	1.3								
7	0.8	0.4	1.3	0.6	1.3	...	0.9				
8	0.8	...	1.2								
9	1.5	...	1.1	0.7							
10	0.6	...	1.0	1.0	0.9						
11	1.2	0.9	2.4	1.0	1.5	...	1.2	...	1.0		
Aver.	1.029	0.68	1.325	0.74	1.08	0.825	1.2	1.3	0.75	0.6	0.8

Some of the results of vaccination on the opsonic index are summarized in the following table:

	25,000,000.	50,000,000.	100,000,000.
Index rose without preceding fall	5	1	4
Index fell with subsequent rise	3	1	4
Index remained unchanged	1	2	1
Index fell and remained depressed	1	1	2

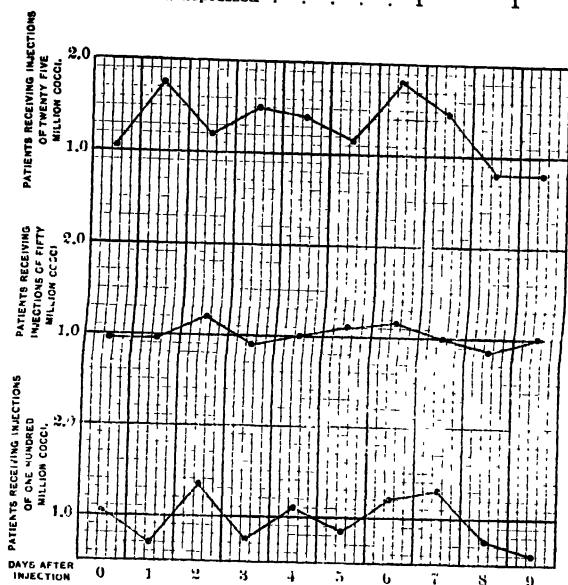


CHART IV.—To show the average effect of vaccination on the opsonic index

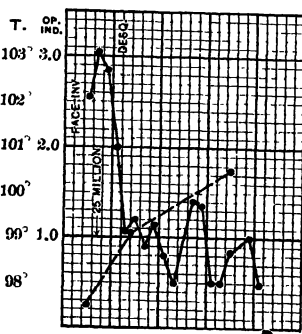


CHART V.—H., an adult male, had been suffering with coryza for about two weeks previous to admission. He had a chill on the day of admission and developed erysipelas of the face. He received one injection of 25,000,000 cocci on the first day of the disease. (Temperature, unbroken line; opsonic index, broken line).

The effect of vaccination on the opsonic index is represented by Chart IV, which shows the average of the opsonic indices for the different days after vaccination with 25,000,000, 50,000,000, and

100,000,000 cocci. Charts V, VI, and VII are from individual cases and show rise of opsonic index after injection of varying amounts of killed streptococci.

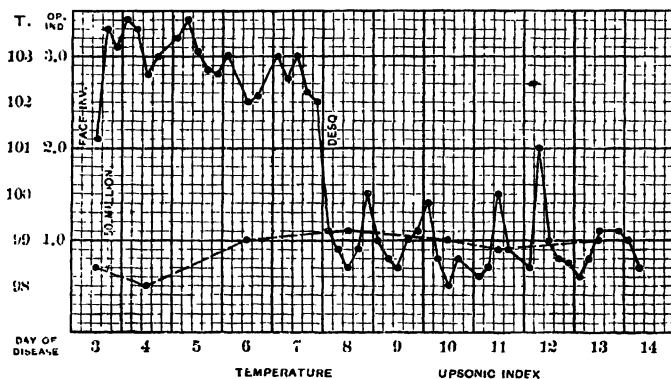


CHART VI.—C., an adult male, suffering with erysipelas of the face and delirium, received an injection of 50,000,000 cocci on the third day of the disease. (Temperature, unbroken line; opsonic index, broken line).

When 25,000,000 cocci are injected, not only is there no negative phase of the opsonic index, but the index is elevated after twenty-four hours. On the day after the first elevation, there is a fall, but the index maintains a level considerably above normal during seven

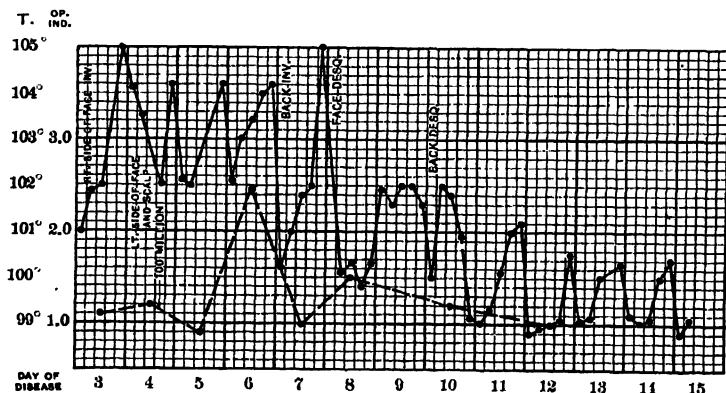


CHART VII.—M., a woman, twenty-three years old, was admitted on the third day of the disease with erysipelas of the right side of the face. Two days before admission she had had a severe chill, felt nauseated and weak. She received 100,000,000 killed cocci on the fourth day of the disease. (Temperature, unbroken line; opsonic index, broken line).

days. Only one-half of the patients discharged from the hospital as cured have a higher index on discharge than on admission.

The opsonic index determined with streptococci from patients with erysipelas shows no constant change at the time of desquamation. At the time of desquamation, the opsonic index was higher

than it had previously been in 8 of 13 cases, and twice it was lower than it had been at any time during the disease.

It is further of interest that of 33 patients whose indices were determined, the indices of 22 of them fell after desquamation. Of these, 2 have been again admitted to the hospital with recurrence.

Of 3 cases of recurrent erysipelas, in 2 there was a lower index at the time of desquamation than there had been before desquamation. One patient had a higher index at the time of the first desquamation and a lower one at the time of the second desquamation than had been observed in the case before.

REPEATED INJECTIONS OF KILLED STREPTOCOCCI. Several cases of migratory erysipelas and several with complications, such as otitis media and pyemia, received more than one injection of killed cultures. In these cases the first injection produced no marked increase of the opsonic index. The second injection was made after an interval of from three to ten days, and was usually smaller than the first. In most cases, the index ultimately rose.

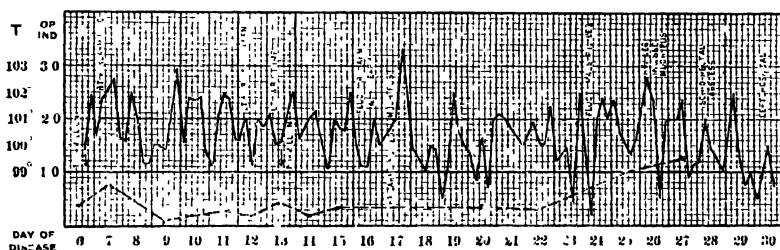


CHART VIII.—F., a woman, thirty-one years old. Five days before admission the patient's right arm began to swell and became red. She felt weak and feverish, and had numerous attacks of vomiting. There was no history of chill or trauma. This patient received an injection of 100,000,000 cocci on the sixth day of the disease, and 50,000,000 cocci on the thirteenth day of the disease. Complications were pyemia and apical tuberculosis. (Temperature, unbroken line; opsonic index, broken line).

In 4 injected patients whose opsonic index was determined at intervals, an intercurrent disease developed. Of these, 2 had pneumonia, 1 pyemia (Chart VIII), and 1 died with the diagnosis of tuberculosis and nephritis (Chart IX). In all of these patients the index rose with the development of the complication. The rise was especially marked with the appearance of pneumonia (Chart X). The significance of this cannot be determined, since only 1 patient with pneumonia died and no autopsy being made, the micro-organism causing the pneumonia could not be determined.

SUMMARY OF CLINICAL RESULTS. *Temperature.* In the first few hours following an injection of killed cultures, there may be a trivial rise of temperature from 0.2° to 0.6° F. There can be no definite conclusions as to the permanent effect on temperature.

Delirium. A large number of the injected patients, as well as those not injected, were delirious at times, so that a conclusion

regarding the relative frequency of delirium after injection is not possible.

Desquamation. Of 21 uncomplicated cases of erysipelas treated by the injection of killed cultures, injections were made twice on the

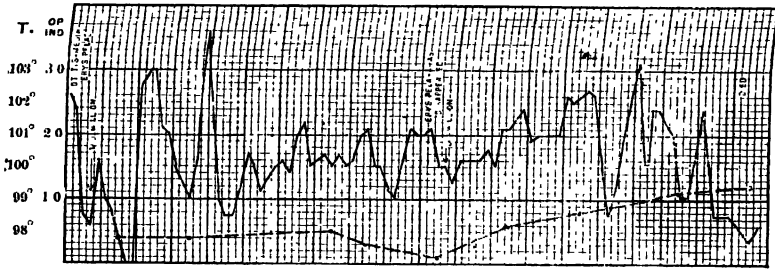


CHART IX.—H., a male, was admitted to the hospital December 3, 1906, with cystitis. On December 27 he developed otitis media, and on January 6 erysipelas of the face. Blood cultures were found to be negative. On January 10 he received 100,000,000 streptococci, again on January 16, 100,000,000, and on January 26, 50,000,000. He was never cured of his otitis media. He died February 5 with a diagnosis of apical tuberculosis and nephritis. (Temperature, unbroken line; opsonic index, broken line)

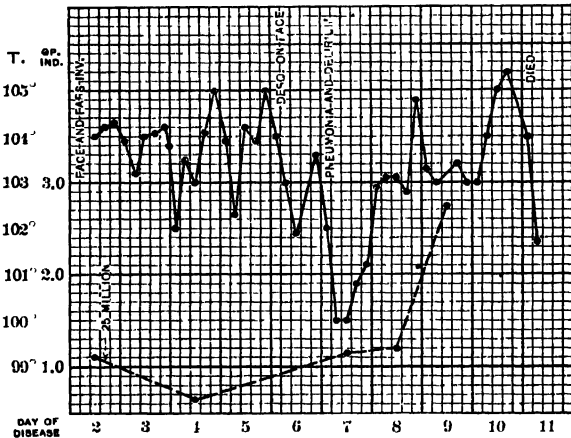


CHART X.—G., a man, thirty-four years old, was admitted on the second day of his disease. On the day before admission the patient had had a severe chill; his face began to burn and became red. He received 25,000,000 killed cocci on the day of admission; on the seventh day the disease was complicated by pneumonia. The patient died on the eleventh day. No autopsy was obtained. (Temperature, unbroken line; opsonic index, broken line).

first day of the disease; these patients desquamated on the second and seventh days, respectively. The average duration from onset to desquamation when treated by injections of antistreptococcic serum, as reported by Dr. Ayer,² in 1905, was seven and one-half days. Two cases of the present series were injected on the second

² Medical Record, 1905.

day of the disease and desquamated on the fourth and sixth days, respectively; an average of 10 cases injected with antistreptococcic serum on the second day by Dr. Ayer desquamated on the seventh day. Four cases injected with killed cultures on the third day of the disease desquamated on the fifth, fourth, fifth, and eighth days, respectively; an average of five and one-half days as compared to an average of six and nine-tenths days, as observed by Dr. Ayer in 21 cases receiving antistreptococcic serum on the third day. Six patients were injected on the fifth day and desquamated on the fifth, eighth, fifth, eighth, eighth, and tenth days of the disease. Two patients were injected on the fifth day of the disease and both desquamated on the seventh day. One patient was injected on the sixth day and desquamated on the seventh day. Three patients were injected on the seventh day and desquamated on the eighth, ninth, and tenth days, respectively. One patient injected on the ninth day of the disease desquamated on the eleventh.

The average duration of the disease in the 21 cases in this series is six and eight-tenth days. In 79 cases of erysipelas which received only local treatment, Dr. Ayer found the average duration of the disease to be nine and four-tenth days, which is two and eight-tenths longer than that of the present series. Of 48 patients receiving antistreptococcic serum, the average duration was found to be about the same as in this series.

The relation of dosage of streptococci to desquamation was as follows: Those receiving 25,000,000 cocci desquamated three days after injection; those receiving 50,000,000 cocci desquamated three and one-half days after injection; and those receiving 100,000,000 cocci desquamated two and four-tenths days after injection.

Migration. Migratory forms were found in 8 patients who had received injections of killed streptococci. Whether this migratory form was caused by injections of cultures cannot be determined, since other cases of the migratory type were observed when no injections were made. One of the injected patients with this form of the disease died; this patient had previously received 60 c.c. of antistreptococcic serum and received one injection of killed cultures of streptococci three days before death. The disease, however, was complicated with chronic interstitial nephritis.

It is quite clear that injections of killed cultures of streptococci from erysipelatos lesions do not prevent migration, even if the opsonic index rises.

Recurrence. Recurrence was observed in a number of cases treated with injections of killed cultures. Since February 15 two of the patients included in this investigation have been again admitted because of recurrence. It is well known that an attack of erysipelas will not prevent a second attack of the disease, hence it is hardly to be expected that the injections of killed cultures will prevent future attacks. Of the 37 patients who received injections

of killed cultures, 3 died. In all of these the erysipelas was complicated by some other disease.

The opsonic index is subject to such great irregularity that its determination gives little indication of the severity of the disease and is of no value for prognosis. There is in most cases of erysipelas an increase of the opsonic index occurring most often during the first three days, and this rise is followed by a fall. The injection of killed streptococci causes, on the whole, an increase of the opsonic index, but a relation between the elevation of the index and the improvement of the patient was not observable.

It is difficult to determine the value of the injection of killed cultures of streptococci in erysipelas. While this injection does not prevent migration and recurrence, the apparent shortening of the duration of the disease suggests that injections have some value. Yet the effect upon the duration of the disease is uncertain because during the time of our investigation no suitable cases remained untreated with which comparison could be made, and it is known that the duration of the disease varies in different years.

This investigation was made possible through the kindness of Dr. J. C. Ayer. I wish to express my obligation to Dr. Ayer and to Dr. Gordon Lindsay, the house officer in Bellevue Hospital.

Zur Kenntnis der Schwefelverbindungen des Nervensystems.

Von

W. Koch.

(Aus dem physiologischen Laboratorium der University of Chicago.)
(Der Redaktion zugegangen am 19. Oktober 1907.)

Mit Ausnahme von Kühne und Chittendens¹⁾ Arbeit über das Neurokeratin und der Beobachtung Kossels,²⁾ daß Protagon Schwefel enthält, liegen über das Studium der Schwefelverbindungen des Nervensystems nur sehr wenige Untersuchungen vor. Selbst die sonst so gründlichen Arbeiten von Thudichum³⁾ berühren kaum dieses Gebiet. Bei meinen quantitativen Untersuchungen des Nervensystems verteilen sich die verschiedenen Schwefelverbindungen nach ihren Löslichkeitsverhältnissen auf vier Gruppen, welche ich mit S_1 , S_2 , S_3 , S_4 bezeichnen werde.

S_1 ; Lipide: Löslich in Alkohol oder Äther oder beiden, unlöslich in 0,5%iger mit Chloroform gesättigter Salzsäure.

S_2 ; Extraktivstoffe: Durch 95%igen Alkohol aus den feuchten Geweben zu entfernen. Löslich in 0,5%iger mit Chloroform gesättigter Salzsäure und auf diese Weise von den Lipoiden zu trennen.

S_3 ; Extraktivstoffe: Durch fünf- bis sechsmalige, jedesmal 24 Stunden andauernde Extraktion mit kaltem Wasser aus den in siedendem Alkohol und Äther unlöslichen Anteil der Gewebe zu erhalten. Es ist besser, bei diesen Extraktionen etwas Chloroform zuzusetzen.

¹⁾ Kühne u. Chittenden, Zeitschrift für Biologie, 1890, Bd. XXVI, S. 291.

²⁾ Kossel und Freytag, Diese Zeitschrift, 1892, Bd. XVII, S. 431.

³⁾ Thudichum, Die chemische Konstitution des Gehirns des Menschen und der Tiere, 1901. F. Piezcker, Tübingen.

S₄; Proteinkörper: Durch andauernde Behandlung mit heißem Alkohol und Äther und sechsmalige Extraktion mit kaltem Wasser von allen anderen Substanzen bis auf einen kleinen Rest anorganischer Substanz befreit.

S₁: Lipoidschwefel.

(Schwefelgehalt des Protagens: 0,88%.)

Bei Versuchen, hier eine Fraktion zu erhalten, welche besonders reich an Schwefel, kam ich auf ein Präparat, das ungefähr die gewöhnlich für das Protagon angegebenen Löslichkeitsverhältnisse besaß. Die Analyse gab folgendes Resultat:

1. 1,000 g Substanz verbrauchten 15,0 ccm $\frac{n}{10}$ -Säure, d. i. 2,1% N
2. 1,500 „ „ „ 21,6 „ „ 2,0% N
3. 0,500 „ „ lieferten 0,0185 g $\text{Mg}_2\text{P}_2\text{O}_7$, d. i. 1,03% P
4. 0,500 „ „ „ 0,0319 „ BaSO_4 , „ 0,88% S

	Gefunden	Cramers ¹⁾ Analysen	Nolls ²⁾ Analysen	Kossels ³⁾ Analysen
N	2,10	2,29	2,57	3,25
P	1,03	1,04	1,15	0,97
S	0,88	0,71	0,65	0,51

Die analytischen Resultate stimmen also auch mit den bis jetzt für Protagone angegebenen annähernd überein. Nur ist zu bemerken, daß mein Präparat aus Menschengehirnen dargestellt, bei welchen nach meinen bisherigen Erfahrungen das Protagon mehr Schwefel enthält. Die bisherigen Untersuchungen über das Protagon von Thierfelder⁴⁾ und Gies⁵⁾ beschäftigen sich hauptsächlich mit dem wechselnden Phosphorgehalt und mit Versuchen, aus dem Präparat reines Cerebrin herzustellen. Cramer betrachtet sein Präparat als einheitlich und bestimmt die Menge des abspaltbaren Cholins, welches nach meiner Berechnung dem Phosphorgehalt ungefähr proportional ist. Um die Zusammensetzung des Protagens annähernd zu bestimmen, habe ich folgende Analysen unternommen.

¹⁾ W. Cramer, Journal of Physiology, 1904, Bd. XXXI, S. 31.

²⁾ Noll, Diese Zeitschrift, 1899, Bd. XXVII, S. 370.

³⁾ Kossel und Freytag, Diese Zeitschrift, 1892, Bd. XVII, S. 431.

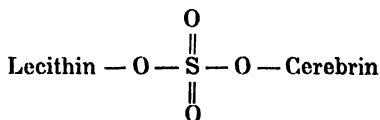
⁴⁾ Thierfelder, Diese Zeitschrift, 1904, Bd. XLIII, S. 21.

⁵⁾ Gies, The Journal of Biological Chemistry, 1905, Bd. I, S. 59.

1. 0,2257 g Substanz mit 1,0%iger Salzsäure 24 Stunden am Rückflußkühler gekocht gaben 0,0120 g BaSO₄, d. i. 0,73% S.
2. 0,300 g Substanz mit 1,0%iger Salzsäure 24 Stunden am Rückflußkühler gekocht reduzierten 0,1123 g CuO, d. i. 16,0 Dextrose.¹⁾
3. 0,300 g Substanz, nach der Methode von Koch und Woods²⁾ auf Lecithin untersucht, gaben 0,0080 g Mg₂P₂O₇, d. i. 0,75% Lecithinphosphor.

	I.	II.	
	Berechnet auf 1 Molekül Lecithin, 3 Moleküle Cerebrin und 1 Molekül Schwefelsäure	Berechnet auf 1 Molekül Lecithin, 3 Moleküle Cerebrin, 1 Molekül Schwefelsäure und 1 Molekül Stickstoff	Gefunden
N	1,63	2,07	2,1
P	0,90	0,89	0,75
S	0,95	0,94	0,73
Dextrose ^{1, 3)}	15,7	15,5	16,0

Die unter II gegebene Berechnung stimmt besser mit der zuerst angegebenen Gesamtanalyse des Protagonen wie mit den hier gefundenen Zahlen überein. Es wird sich auch wohl kaum um einen einheitlichen Körper handeln. Die recht nahe Übereinstimmung mit den molekularen Verhältniszahlen des Lecithinphosphors und des als Schwefelsäure abgespaltenen Schwefels deutet jedoch auf die womögliche Existenz der folgenden Verbindung:



Eine derartige Verbindung würde eine einfache Erklärung für die wohlbekannte Tatsache geben, daß Lecithin sich nicht mit kaltem Alkohol oder Äther, in welchen es im reinen Zustande leicht löslich ist, aus Protagon entfernen läßt. Daß die Schwefelsäure wirklich in esterartiger Verbindung mit dem

¹⁾ Wegen der Ungenauigkeit der Zahlen für Galaktose als Dextrose berechnet.

²⁾ Koch und Woods, Journal of Biological Chemistry, 1905, Bd. I, S. 203.

³⁾ Mit Zugrundelegen von Thierfelders Bestimmung berechnet.

Cerebrin sich befindet, geht daraus hervor, daß sich aus dem Präparat durch Behandlung mit Chloroform gesättigter 5%iger Salzsäure in der Kälte nur minimale Mengen Schwefelsäure gewinnen lassen (aus 1,5 g nur 0,003 g BaSO_4).

Aus der mehr salzartigen Verbindung mit Lecithin könnte die Schwefelsäure wohl eher auf diese Weise entfernt werden. Nimmt man an, daß der reduzierende Zucker nur aus Cerebrin stammt, so ergibt die Berechnung mehr Cerebrin, wie in obiger Verbindung vorhanden sein kann. Der Überschuß ist wohl auf Gamgees Pseudocerebrin oder Thierfelders Cerebron zu beziehen. Wie mir nun Dr. Levene persönlich mitteilt, läßt sich im Protagon mit der Orcinprobe eine Pentose nachweisen. Die Verhältnisse liegen also ziemlich verwickelt und das Studium der Zusammensetzung des Protagongemisches scheint mir daher zurzeit wichtiger als langwierige Untersuchungen darüber, ob ein einheitlicher Körper vorliegt oder nicht.¹⁾ Obgleich mein Präparat weniger Stickstoff und mehr Schwefel enthält, wie man bisher gefunden, ist mir die Darstellung der Verbindung von Lecithin und Cerebrin mit Schwefelsäure noch nicht gelungen. Auch müssen noch weitere Untersuchungen darüber entscheiden, welchem Anteil des Protagons der Überschuß von einem Molekül Stickstoff zukommt.

Interessant ist, daß, wie schon Noll für das Cerebrin angegeben, auch der hier untersuchte Schwefel, den ich von jetzt ab als Lipoidschwefel bezeichnen werde, vorwiegend in der weißen Substanz vorhanden, wie aus folgenden Zahlen ersichtlich.

Lipoidschwefel in Prozent der Trockensubstanz berechnet	
Muskel	0,008
Submaxillardrüse	0,018
Hoden	0,023
Leber	0,036
Graue Nervensubstanz	0,040
Weißer » (corpus callosum)	0,180

Weitere schwefelhaltige Körper aus den Lipoiden zu gewinnen, ist mir noch nicht gelungen, obgleich alle Präparate,

¹⁾ W. Cramer und H. C. Lockhead M. C., The Biochemical Journal, 1907, Bd. II, S. 355.

sogar Cerebrin, wenn nicht besonders sorgfältig gereinigt, etwas Schwefel enthalten, was aber wohl auf Verunreinigungen zu beziehen ist. Der im Protagogengemisch vorhandene Schwefelkörper wird wohl der einzige Lipoidschwefelkörper sein, denn das Verhältnis von Schwefel zu Cerebrin berechnet sich im Corpus callosum beinahe genau so wie im Protagon.

Schwefel : Cerebrin = 1 : 86 im corpus callosum

„ : „ = 1 : 83 „ Protagon.

S_2 : Neutralschwefel.

1. *Anorganische Sulfate.*

2. *Taurinartige Schwefelverbindung.*

Diese Gruppe enthält ungefähr ein Zehntel ihres Gesamtschwefels in Form anorganischer Sulfate, welche sich mit Baryumchlorid direkt bestimmen lassen. Im Filtrat vom Baryumsulfat befindet sich der bei weitem größere Teil des Schwefels, welcher sich jedoch selbst nach andauerndem Kochen mit einprozentiger Salzsäure nicht als Baryumsulfat gewinnen läßt. Setzt man dieser Lösung Phosphorwolframsäure zu, so entsteht ein Niederschlag, welcher aber keinen Schwefel enthält. Im Filtrat, welches durch Baryumhydrat vom Überschuß der Phosphorwolframsäure befreit, befindet sich ein Körper, welcher mit Naphthylisocyanat reagiert. Durch anhaltende Behandlung mit Naphthylisocyanat kann die Lösung beinahe ganz von Schwefel befreit werden. Die Naphthylisocyanatverbindung enthält 0,8% Schwefel und gibt keine Orcinreaktion. Obgleich es mir hier ebenfalls nicht gelungen ist, einen einheitlichen Körper darzustellen, glaube ich doch annehmen zu sollen, daß es sich um ein Gemisch von Monoaminosäuren handelt. Die Eigenschaften des Schwefelkörpers würden am besten mit denen des Taurins oder einer komplizierten Vorstufe desselben übereinstimmen. Der Übersicht halber werde ich von jetzt ab S_2 als die Neutralschwefelgruppe bezeichnen. Cystinartiger oder bleischwärzender Schwefel läßt sich aber nicht nachweisen.

S_3 : Anorganische Sulfate.

Enthält außer den anorganischen Sulfaten noch proteinähnliche Schwefelverbindungen (Gelatine?)

Diese Gruppe enthält sehr wenig organische Substanz, nie mehr als ein Prozent der Gesamttrockensubstanz des Gehirns. Wahrscheinlich handelt es sich um proteinartige Körper, welche durch die Alkoholbehandlung in Wasser nicht ganz unlöslich gemacht worden sind. Mit Phosphorwolframsäure läßt sich aus der Lösung ein ungefähr 0,3% Schwefel enthaltender Körper in geringer Menge gewinnen. Durch Behandlung mit heißem Wasser ist es mir gelungen, aus einer größeren Menge Gehirnschubstanz nach vorheriger Behandlung mit Alkohol und Äther einen gelatineartigen Körper zu erhalten, welcher Millons Reaktion, aber nicht die Reaktion auf Tryptophan gibt. Über zwei Drittel des Schwefels dieser Gruppe läßt sich durch Baryumchlorid direkt als anorganisches Sulfat nachweisen.

S₄: Proteinschwefel.

1. Schwefelgehalt des Neurokeratins: 1,60—2,24%.
2. Schwefelgehalt des Nucleoproteins: 1,29%, Levene,¹⁾ Halliburton.²⁾
3. Globulin koaguliert bei 47°—50° C. } Schwefelgehalt
4. " " " 70° C. } nicht bestimmt.

Das Neurokeratin wurde zuerst von Kühne und Chittenden eingehender studiert und seine Ähnlichkeit mit den aus Horn gewonnenen Keratinen klargelegt. Am Stoffwechsel des Nervensystems wird es sich wohl kaum beteiligen, da es hauptsächlich in den markhaltigen Fasern die Rolle einer Gerüstsubstanz versieht.

Das Nucleoprotein, welches Levene studiert, ist nach Halliburton³⁾ mit seinem bei 57° C. koagulierenden Nucleoprotein identisch. Halliburton²⁾ gibt an, daß sich dieser Körper nur in der grauen Substanz befindet und nicht aus

¹⁾ P. S. Levene, Archiv of Neurology and Psychopathology, 1899, Bd. VII, S. 14.

²⁾ Halliburton, Collected papers from the Physiological Laboratory of King's College London, 1893, Nr. 1. Siehe auch: British Medical Journal, 1893. Goulstonian Lectures.

³⁾ Halliburton, Ergebnisse der Physiologie, 1905, Bd. IV, S. 31.

weißer Substanz gewinnen läßt. Interessant ist, wie später bei meiner Berechnung der Verteilung des Proteinschwefels ersichtlich, daß das Nucleoprotein im corpus callosum beinahe gar keinen Schwefel enthält, während nach Levenes und meinen Berechnungen das Nucleoprotein aus der grauen Substanz verhältnismäßig reich daran ist. Es wird sich also im Gehirn um mindestens zwei Nucleoproteine handeln: ein in der grauen Substanz befindliches schwefelhaltiges und ein sowohl in der grauen wie in der weißen Substanz in den Kernen der Gliazellen befindliches, schwefelarmes. Letzteres Nucleoprotein ist meines Wissens bis jetzt noch nicht isoliert worden. Ob das erstere von Levene und Halliburton studierte mit der Nisslsubstanz in irgend welcher Beziehung steht, was ja nach dem Verhalten der Nisslsubstanz zu basischen Farbstoffen nicht unmöglich, ist bis jetzt noch nicht untersucht. Interessant ist hier die Beobachtung von Mott,¹⁾ welcher findet, daß bei der amaurotischen Idiocie, mit einem Verschwinden der Nisslsubstanz, eine Verminderung des Nucleoproteinphosphors der grauen Substanz parallel verläuft. Die Globuline von Halliburton sind bis jetzt noch nicht auf ihren Schwefelgehalt untersucht worden.

Obgleich es mir noch nicht gelungen ist, wegen der Schwierigkeit in der Beschaffung von Menschengehirnen, welche sich zu diesen Arbeiten am besten eignen, einen wirklich reinen Schwefelkörper herzustellen, ist es doch interessant, die quantitative Verteilung des Schwefels auf die verschiedenen Gruppen zu vergleichen. Die in folgender Tabelle angegebenen analytischen Resultate wurden an einem vollkommen normalen, frischen, beinahe blutfreien Gehirn eines neunzehnjährigen Mannes gewonnen, welcher an Verblutung aus der Art. carotis interna gestorben war. Das Gehirn wurde mir von meinem Kollegen Dr. H. G. Wells gütigst zur Verfügung gestellt. Die vollkommene chemische Analyse wird demnächst an anderer Stelle erscheinen.

¹⁾ F. W. Mott, Archives of Neurology, 1907, Bd. III, S. 244.

	Graue Rinden- substanz		Corpus callosum	
	In Prozent der Trocken- substanz	In Prozent des Gesamt- schwefels	In Prozent der Trocken- substanz	In Prozent des Gesamt- schwefels
S ₁ Lipoid	0,033	7,2	0,180	35,9
{ S ₂ Neutral	0,050	10,9	0,025	5,0
{ S ₂ Sulfate	0,007 }	5,9	0,006 }	3,4
{ S ₃ Sulfate	0,020 }		0,011 }	
{ S ₃ Proteinähnlich . .	0,013	2,8	0,023	4,6
{ S ₄ Globulin ¹⁾	0,125	27,2	0,040	8,0
{ S ₄ Nucleoprotein ²⁾ . .	0,166	36,0		
{ S ₄ Neurokeratin ³⁾ . .	0,046	10,0	0,216	43,2
	0,460		0,501	
Gesamtschwefel	0,430		0,420	
(Kontrollbestimmung)				

Die obigen Zahlen deuten darauf hin, daß:

in der grauen Substanz Nucleoprotein, Globulin und Neutralschwefel vorherrschen;

in der weißen Substanz bei weitem der größte Anteil auf Neurokeratin und Lipoidschwefel zu beziehen ist.

Das in der grauen Substanz vorhandene Neurokeratin und der Lipoidschwefel stehen ungefähr im selben relativen Verhältnis zu einander wie im corpus callosum und es scheint daher die Annahme berechtigt, daß es sich bei beiden um charakteristische Bestandteile der markhaltigen Fasern handelt. Ganz frei von weißer Substanz läßt sich ja bekanntlich graue Substanz nicht gewinnen.

¹⁾ Aus der Differenz zwischen Nucleoprotein- plus Neurokeratinschwefel und dem direkt bestimmten Gesamtproteinschwefel berechnet.

²⁾ Aus meiner Proteinphosphorbestimmung auf Grund von Levenes Schwefelbestimmung berechnet. Das Resultat wird etwas zu hoch sein, da nicht alles Nucleoprotein in der grauen Substanz mit der von Levene studierten Substanz identisch ist.

³⁾ Aus Chittendens analytischen Befunden an einem 21jährigen Manne annähernd berechnet.

Ehe ich auf die Bedeutung der obigen Befunde für die Erklärung des Stoffwechsels des Nervensystems näher eingehe, scheint es angebracht, unsere bisherigen Kenntnisse über dieses interessante Gebiet zusammenzustellen.

Stellen wir uns ein lebendes Gewebe nicht als eine Anzahl sogenannter lebender Moleküle, sondern als den Ort der Zusammenwirkung mehrerer chemischer Reaktionen vor, so handelt es sich darum, die einzelnen Reaktionen zu erkennen und zu studieren. Dies kann man nun auf dreierlei Weise erreichen.

1. Am lebenden Gewebe, wie dies Hill¹⁾ bei seinen Untersuchungen über die CO₂-Produktion des Nervensystems getan.

2. Am überlebenden Gewebe wie bei den Arbeiten von Hofmeister²⁾ über Methylstoffwechsel und zahlreichen Arbeiten anderer Forscher über Autolyse.

3. An toten Geweben, wie bei meinen hier zu schildernden Versuchen, durch das Studium der unter den Extraktivstoffen befindlichen Stoffwechselprodukten des Gehirns in normalen und pathologischen Fällen.

Obgleich nun der geringe Gewichtsverlust des Nervensystems beim Hungern die von mehreren Forschern beobachtete sehr langsam verlaufende Autolyse, sowohl wie die oben erwähnten Beobachtungen von Hill und Hofmeister darauf hindeuten, daß im Nervensystem die chemischen Reaktionen sehr langsam verlaufen, beweisen die Versuche von Ehrlich,³⁾ Hill,¹⁾ Baeyer,⁴⁾ Bondy⁵⁾ und Mott und Sherrington,⁶⁾ daß das Gehirn fortwährend mit einem großen Sauerstoffüberschuß versorgt sein muß, um normal zu funktionieren. Nun

¹⁾ L. Hill, *Journal of Physiology*, 1895, Bd. XVIII, S. 334.

²⁾ Hofmeister, *Archiv für experimentelle Pathologie und Pharmakologie*, 1894, Bd. XXXIII, S. 198.

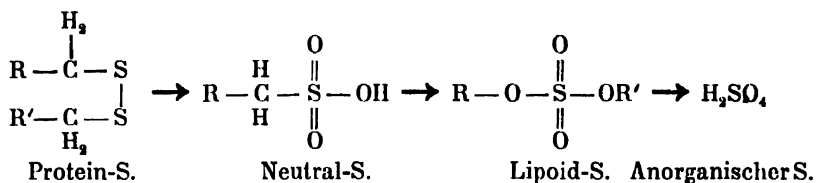
³⁾ P. Ehrlich, *Sauerstoffbedürfnis des Organismus*.

⁴⁾ H. v. Baeyer, *Zeitschrift für allgemeine Physiologie*, 1902, Bd. I, S. 265.

⁵⁾ Bondy, O., *Zeitschrift für allgemeine Physiologie*, 1903, Bd. III, S. 180.

⁶⁾ Mott u. Sherrington, *Croonian Lectures*, London, 1900, S. 50.

bedingt aber eine gute Sauerstoffversorgung nicht zugleich einen großen Sauerstoffverbrauch, sondern es mag sich lediglich um das Bedürfnis einer hohen Sauerstoffspannung handeln. So deuten die Beobachtungen von Baeyer bei der Strychninvergiftung darauf hin, daß nur bei Gegenwart eines genügenden Sauerstoffpotentials die Entladung der Nervenzelle stattfinden kann. Bei welcher Art Reaktionen sich dieser Sauerstoff beteiligt, wissen wir noch nicht. Unter anderem wird es sich wohl auch um die Oxydation von Proteinschwefel handeln, welches den oben angegebenen Tatsachen entsprechend ungefähr folgendermaßen verlaufen würde.



Lipoidschwefel braucht natürlich nicht in jedem Falle gebildet zu werden, sondern es kann sich auch um direkte Oxydation von Neutralschwefel zu anorganischen Sulfaten handeln.

Es ist nun schon oft, besonders von Kraepelin, die Ansicht ausgesprochen worden, daß es sich bei gewissen Geisteskrankheiten um einen gestörten Stoffwechsel des Nervensystems handeln kann. Da es nun gerade Oxydationsreaktionen wie die oben angedeuteten sind, bei welchen man unter pathologischen Verhältnissen der Gewebe am ersten Veränderungen erwarten kann, nahm ich gerne die Gelegenheit wahr, welche mir von Dr. F. W. Mott, F. R. S. angeboten wurde, dieses Thema an Fällen von Dementia praecox im Pathologischen Institut der London County Asylum zu studieren. Über die klinische und histologische Untersuchung der Fälle wird an anderer Stelle berichtet (Archives of Neurology, F. W. Mott).

In folgender Tabelle sind die analytischen Resultate der grauen Rindensubstanz von vier Fällen angegeben. Zum Vergleich füge ich die Zahlen von drei normalen Gehirnen bei. Die Zahlen sind in Prozent des Gesamt-Nicht-Proteinschwefels

berechnet. Es ist von Interesse, hier zu erwähnen, daß diese Fälle von Dementia praecox, was alle anderen bekannten Bestandteile des Nervensystems anbelangt, bei der chemischen Analyse Resultate ergaben, welche vom Normalen nicht zu unterscheiden sind.

In Prozent des Gesamt-Nicht-Proteinschwefels der grauen Rindensubstanz.

Fall Nr.	Lipoid-S ₁	Neutral-S ₂	Anorganische Sulfate S ₃
7. K. R. Normal	27	51	21
14. E. McC. »	24	42	33
15. R. A. G. »	27	46	27
Durchschnitt . .	26	46	27
10. M. A. N. Dementia praecox	27	30	44
11. C. E. N. » »	16	35	49
12. F. L. M. O. » »	17	34	48
17. H. F. R. » »	40	22	38
Durchschnitt . .	25	30	44

Zuerst fällt bei allen Fällen der Dementia praecox eine Verringerung des Neutralschwefels auf (35% im Durchschnitt). Die höhere Zahl für anorganische Sulfate beruht nicht, wie wohl zuerst anzunehmen, auf einer gesteigerten Oxydation von Neutralschwefel.

Bei 17. H. F. R. handelt es sich bei dieser Art der Berechnung um eine relative Erhöhung der Zahl für Lipoid- und anorganischen Schwefel, wegen des Verlustes an Neutralschwefel. Bei 11. C. E. N. und 12. F. L. M. O. kommt dann noch eine tatsächliche Verminderung des Lipidschwefels hinzu, aus welchem ja anorganische Sulfate ohne Oxydation entstehen können. Bei Fall 10. M. A. N. läßt sich die höhere Zahl für anorganische Sulfate auf diese Weise nicht ganz erklären. Die analytischen Resultate für das corpus callosum zeigen eine bedeutend geringere Veränderung in den pathologischen Fällen.

Es tritt also ziemlich klar zutage, daß es sich bei der Dementia praecox um eine gestörte Oxydation handelt und zwar auf Kosten des intermediär gebildeten Neutralschwefels.

Ich bin jetzt dabei, diese Beobachtungen an weiteren Fällen nachzuprüfen und zu erörtern, inwieweit die Lungentuberkulose, an welcher alle Fälle gestorben sind, für die chemischen Veränderungen verantwortlich zu machen ist. Eine gewisse Bestätigung finden obige Beobachtungen in der Untersuchung eines sechs Wochen alten Gehirns, in welchem man wegen des raschen Wachstums in diesem Alter einen gesteigerten Stoffwechsel erwarten kann.

Fall Nr.	Lipoid-S ₁	Neutral-S ₂	Anorganische Sulfate S ₃
13. S. H.	16	68	16

Es findet sich also gerade die Schwefelgruppe vermehrt, welche bei der Dementia praecox verringert ist.

Über den Umfang des durch obige Beobachtungen sehr wahrscheinlich gemachten Schwefelstoffwechsel des Nervensystems läßt sich nichts Bestimmtes aussagen. Untersuchungen über Schwefelausscheidung im Urin bei geistiger Tätigkeit dürften wohl ebensowenig Aussicht auf Erfolg haben, wie die bisherigen Versuche, die Phosphorausscheidung auf ähnliche Weise zu beeinflussen. Es wäre unrichtig, diesen Stoffwechsel als für das Nervensystem charakteristisch anzusehen, denn in ihren chemischen Reaktionen werden sich wohl die verschiedenen Gewebe des Körpers viel näher stehen, wie in ihrem anatomischen Aufbau.

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THE EFFECT OF CERTAIN SURGICAL ANTISEPTICS AND THERAPEUTIC AGENTS ON PHAGOCYTOSIS.

I. CARBOLIC ACID, MERCURIC CHLORIDE, BORIC ACID, QUININE HYDROCHLORIDE.¹

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The present wide interest in opsonotherapy and belief in the importance of phagocytosis in immunity, make it desirable to determine the effect of the commoner medicinal agents on phagocytic power. The effect of the three most important surgical antiseptics, namely, mercuric chloride, carbolic acid and boric acid, and of the most widely used internal remedy in acute infections, quinine hydrochloride, have thus far been determined, and are here presented.

Material and Technique.—Except in the one experiment herein-after specially mentioned, the material used in this study was defibrinated human blood and streptococci suspended in physiological saline (0.85 per cent. sodium chloride). The streptococci were in all cases obtained from twenty-four to forty-eight hour cultures on blood serum (goat), sterilized at 100° C.

To test the effect of a chemical substance on phagocytosis, increasing amounts of a solution of that substance (whenever possible equimolecular with 0.85 per cent. sodium chloride) were added to a number of test-tubes, and the volumes in the tubes made constant (1 c.c.) with physiological saline. There was then added to each tube a constant amount (1 c.c.) of freshly drawn, defibrinated blood and, at stated intervals, a constant volume (1 c.c.) of streptococcus suspension.

Each tube was immediately immersed in a thermostatic water bath at 37.5° C., and fifteen, thirty and sixty minutes later, smears

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were made from it. These smears were stained by Wright's method and the number of bacteria in sixty polymorphonuclear leucocytes in each smear counted.

Experimental Error.—In beginning quantitative work in any field of biological chemistry, it is necessary, first of all, to determine the accuracy of the proposed experimental method—the limits of the experimental error. Without such determination, deductions from experimental data are comparatively valueless. An experiment was therefore undertaken to determine the range of error in work by the above technique.

To do this, ten duplicate tubes were prepared, each containing a mixture of equal parts (1 c.c.) of physiological saline, defibrinated human blood and streptococcus suspension. Smears were made from these tubes and counted, exactly as in the proposed investigation.² The data from this experiment are given in Table I.

TABLE I.

Duplicate Bacterial Counts.

		1	2	3	4	5	6	7	8	9	10	Average.
15 Minute Counts	Wright's Index	5.63	5.58	6.18	5.23	5.87	5.83	5.72	5.27	5.53	5.21	5.61
	Error	+ .02	— .03	+ .57	— .38	+ .26	+ .22	+ .11	— .34	— .08	— .40	± .24
	Percentage Error	+ 0.4	— 0.5	+ 10.0	— 6.8	+ 4.6	+ 3.9	+ 2.0	— 6.1	— 1.4	— 7.1	± 4.3
30 Minute Counts	Wright's Index	[9.63]	8.40	7.65	7.48	7.38	7.35	7.88	8.15	7.83	7.50	7.73
	Error	[+1.90]	+ .67	— .08	— .25	— .35	— .38	+ .15	+ .42	+ .10	— .23	± .29
	Percentage Error	[+24.6]	+ 8.7	— 1.0	— 3.2	— 4.5	— 4.9	+ 1.9	+ 5.4	+ 1.3	— 3.0	± 3.8
60 Minute Counts	Wright's Index	12.60	11.80	12.15	12.05	11.93	11.35	11.48	10.87	11.03	11.08	11.64
	Error	+ .96	+ .24	+ .51	+ .41	+ .29	— .29	— .16	— .77	— .61	— .56	± .48
	Percentage Error	+ 8.2	+ 2.1	+ 4.4	+ 3.5	+ 2.5	— 2.5	— 1.4	— 6.6	— 5.2	— 4.8	± 4.1

From this table it is seen that the average error in the fifteen minute counts is 4.3 per cent., with a maximum error of 10 per cent. In the thirty minute counts, with the exception of a single smear, the average error is 3.8 per cent., and the maximum 8.7 per

² This experiment, of course, was not attempted till the technique had been mastered by preliminary work.

cent., the one exceptional smear giving an error of 24.6 per cent. In the sixty minute counts, the average error is 4.1 per cent., the maximum 8.2 per cent.

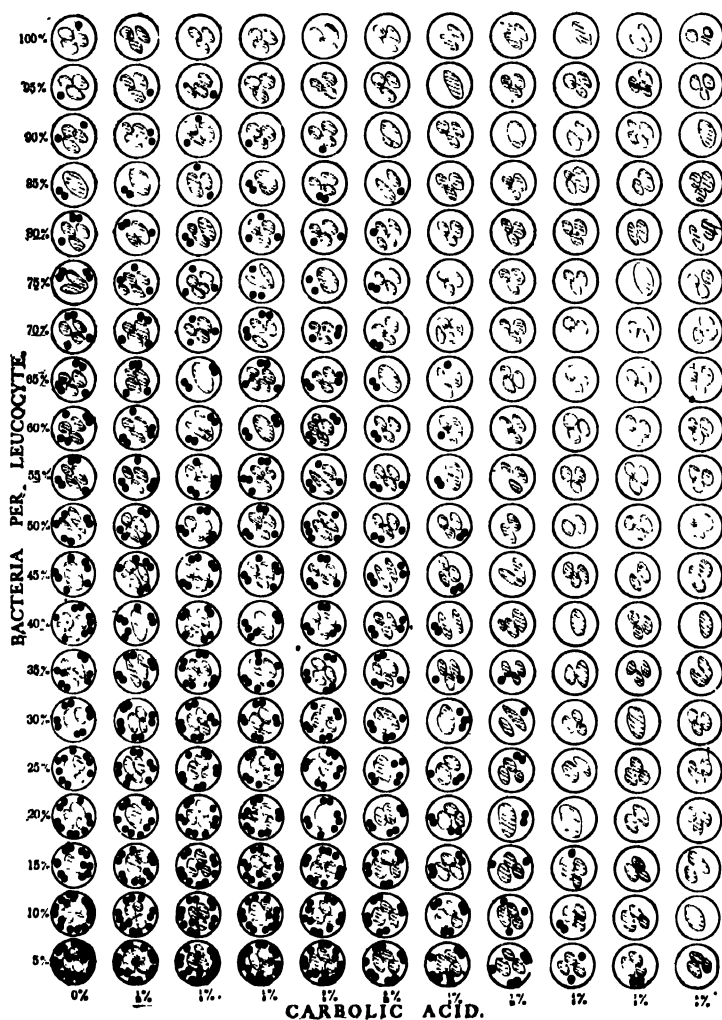


FIG. 1. Influence of carbolic acid on phagocytosis. Human leucocytes; average of 15, 30 and 60 minute counts.

In interpreting data obtained by the above technique, therefore, allowance must be made for a usual maximum error of about 10 per cent., but an occasional error (one count in thirty) of as much

as 25 per cent. must be expected. To eliminate this large experimental error, the average of a large number of data must be used.

Graphic Representation.—In recording data in most fields of quantitative chemistry, it is desirable to select a graphic method of representation. Such a method for the phenomenon of phagocytosis, must give not only the average number of the bacteria per leucocyte, and the number of leucocytes ingesting bacteria, but the numerical distribution of the bacteria among the different leucocytes, as well.

The graphic method selected is shown in the column "0%" of Fig. 1. This column consists of twenty diagrammatic leucocytes,

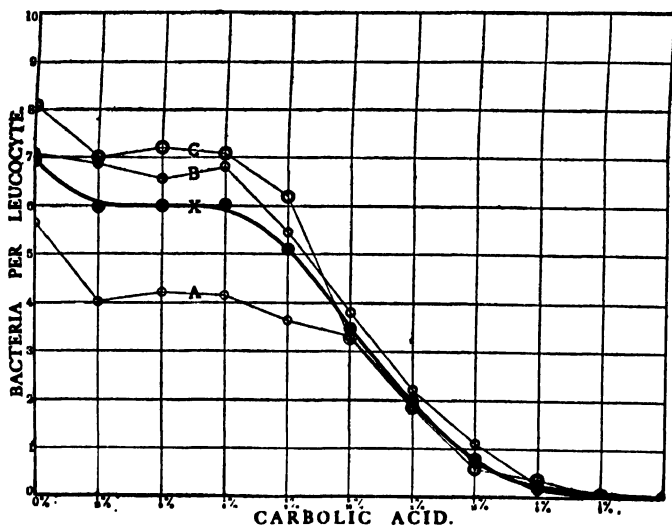


FIG. 2. Influence of carbolic acid on Wright's index. Data from Figure 1, A=15-minute counts; B=30-minute counts; C=60-minute counts; X=average.

each leucocyte representing 5 per cent. of the polymorphonuclear neutrophils present in a given smear. In each leucocyte is marked the average number of bacteria found in its corresponding 5 per cent.

Carbolic Acid.—The average of the three counts obtained by the above technique, with carbolic acid, gives the data recorded in Fig. 1. These data can be translated in terms of the currently used

Wright's index, by calculating the average number of bacteria taken up per leucocyte. Such calculations are shown graphically by the broken lines *A*, *B* and *C* of Fig. 2. The average of these observa-

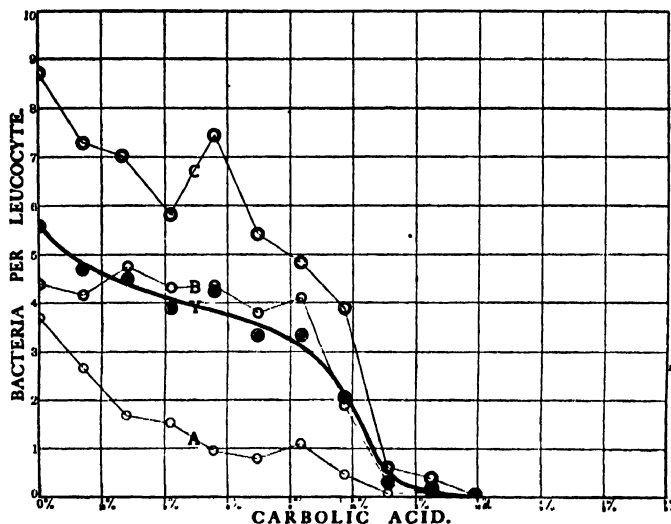


FIG. 3. Influence of carbolic acid on Wright's index. Rabbit leucocytes. *A*, *B* and *C* = 15-minute, 30-minute and 60-minute counts; *X* = average.

tions, plotted as smooth curves to eliminate experimental errors, as is customary in physical experimentation, is given in the heavy

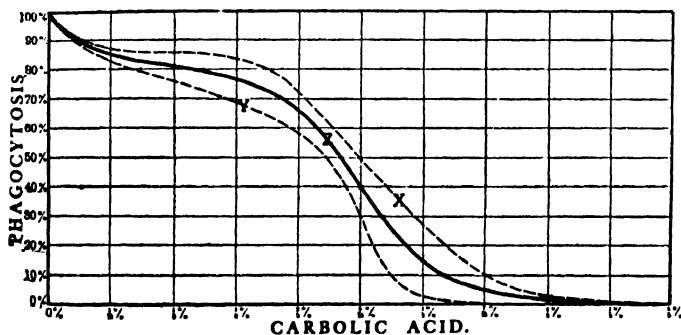


FIG. 4. Influence of carbolic acid on Wright's index; Curves *X* and *Y*, of Figs. 2 and 3, plotted to the same scale. *Z* = average.

curve *X*. A similar curve, obtained with rabbit blood in place of human blood, is given in Fig. 3. The curves of these two experi-

ments, reduced to percentage curves, by taking the phagocytosis in the control tubes of each experiment as 100 per cent., are shown in Fig. 4.

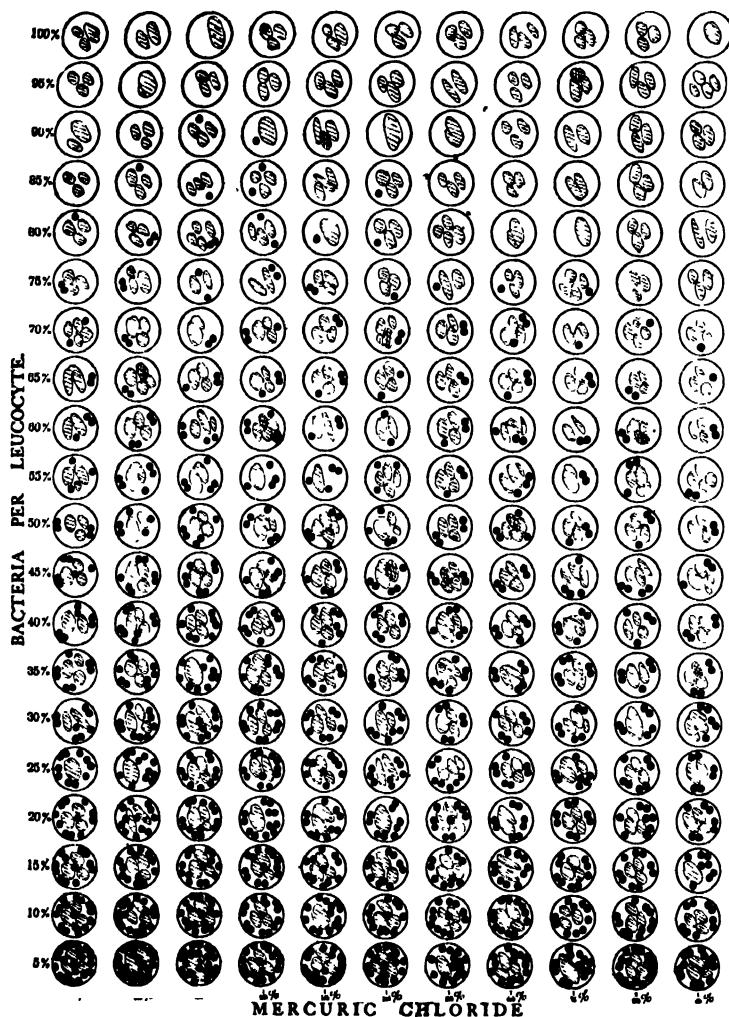


FIG. 5. Influence of mercuric chloride on phagocytosis. Human leucocytes; average of 15- and 30-minute counts.

From this figure, it is seen that the addition of carbolic acid to the experimental tubes causes, from the first, a decrease in phagocytic power, phagocytosis falling off about one third by the time the

carbolic acid reaches a concentration⁸ of 2/9 per cent. A further increase in carbolic acid causes an almost precipitous fall in phagocytic power, phagocytosis being reduced seven eighths by the time

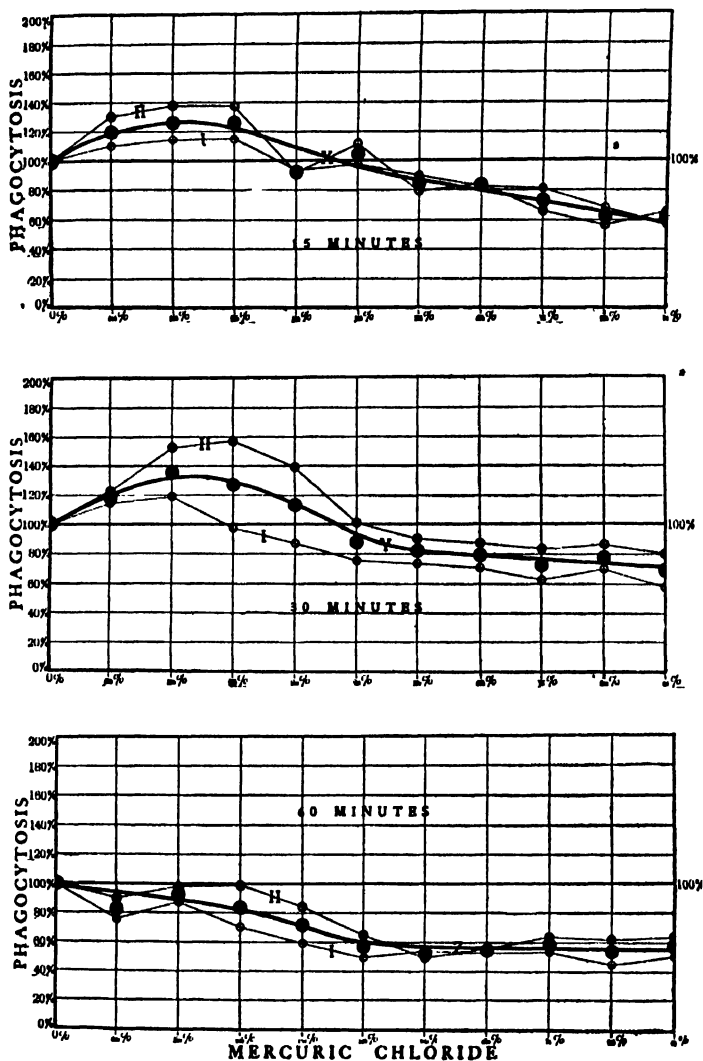


FIG. 6. Influence of mercuric chloride on Wright's index. 15, 30- and 60-minute data plotted separately. I = data from first experiment; II = data from second experiment; X, Y and Z = averages.

⁸ Concentration measured in terms of final dilution.

the concentration reaches one third per cent., and entirely ceasing at one half per cent.

Mercuric Chloride.—The average of the fifteen and thirty minute counts, in two experiments with mercuric chloride are given in Fig. 5. Since the sixty minute counts and the earlier counts in these experiments show different effects of mercuric chloride, the influence of this antiseptic on phagocytosis can not be represented by a single curve, as was done with carbolic acid. Three percentage curves were therefore plotted, showing the changes in Wright's index, at the end of fifteen, thirty and sixty minutes. These curves are shown in Fig. 6. In each curve 100 per cent. stands for the

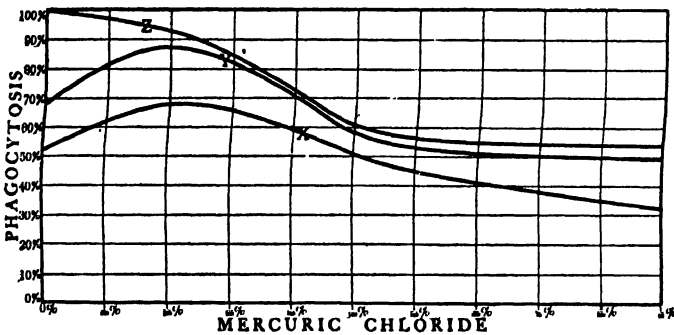


FIG. 7. Influence of mercuric chloride on Wright's index. Curves of Fig. 6 plotted to the same scale.

phagocytosis in the control tubes, containing no antiseptic.

These three curves may be reduced to the same scale by taking the normal sixty minute phagocytosis as 100 per cent. Thus reduced, they are shown in Fig. 7. From this figure, it is seen that mercuric chloride causes, in small amounts, a preliminary stimulation in phagocytosis, followed by a depression. The maximum preliminary stimulation is obtained with a bichloride concentration of 1/300 per cent. As the concentration increases beyond this amount, the preliminary stimulation decreases, and completely disappears at 1/120 per cent.

A further increase in mercuric chloride causes a pronounced fall in phagocytic power, early phagocytosis being reduced 50 per cent. by the time the concentration reaches 1/60 per cent. With con-

centration of 1/200 per cent. and over, phagocytosis practically ceases at the end of thirty minutes.

Boric Acid.—The average of the fifteen and thirty minute counts

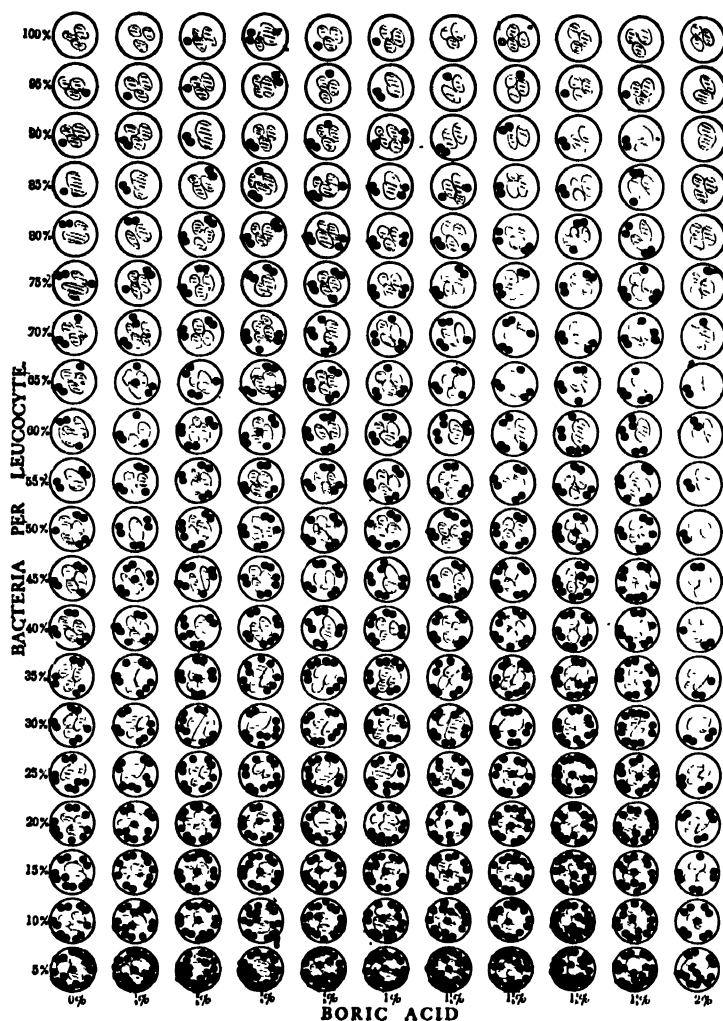


FIG. 8. Influence of boric acid on phagocytes. Human leucocytes, average of 15- and 30-minute counts.

in two experiments with boric acid are given in Fig. 8. With boric acid, as with mercuric chloride, the sixty minute counts

and the earlier counts show different effects on phagocytosis. Three percentage curves were therefore plotted (Fig. 9) showing the observed effect at the end of fifteen, thirty and sixty minutes. These curves are plotted to the same scale as given in Fig. 10.

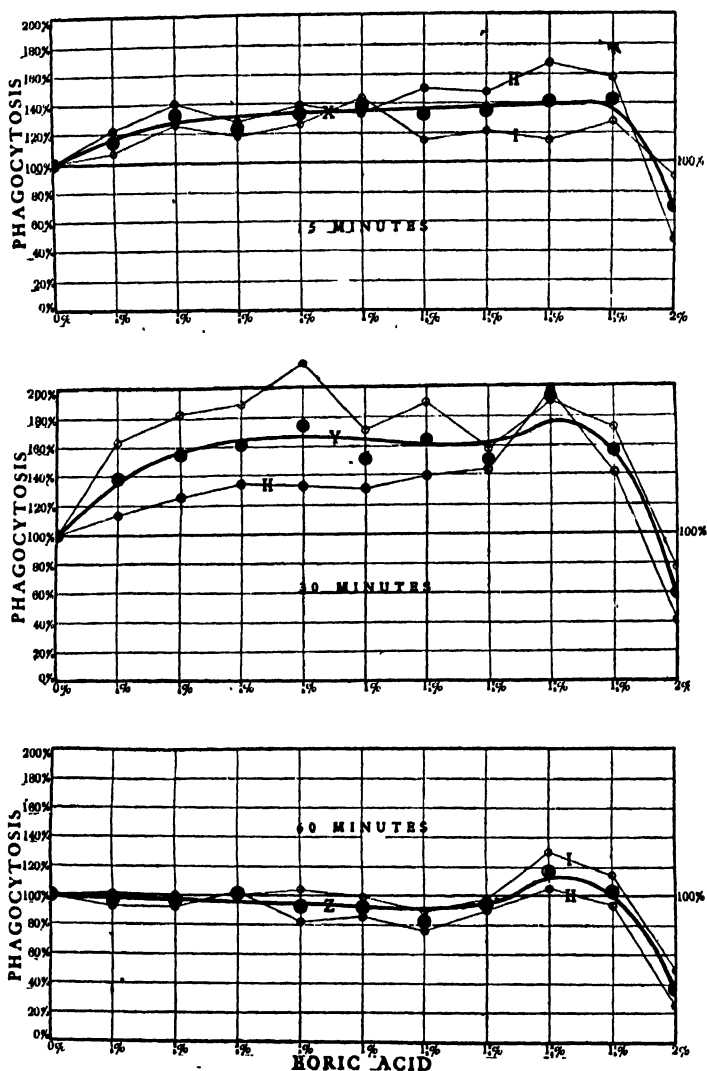


FIG. 9. Influence of boric acid on Wright's index. 15-, 30- and 60-minute data, plotted separately. *I* = data from first experiment, *II* = data from second experiment; *X*, *Y* and *Z* = averages.

From Fig. 10 it is seen that boric acid, in small amounts, causes a preliminary stimulation in phagocytosis, followed by a depression. This phenomenon becomes more marked as the percentage of boric acid increases, until a concentration of $1\frac{1}{2}$ per cent. is reached. After this a further increase in boric acid causes a rapid fall in phagocytic power, phagocytosis apparently completely ceasing soon after the concentration reaches 2 per cent.

Quinine Hydrochloride.—The average of the three counts in an experiment with quinine hydrochloride are given in Fig. 11, and

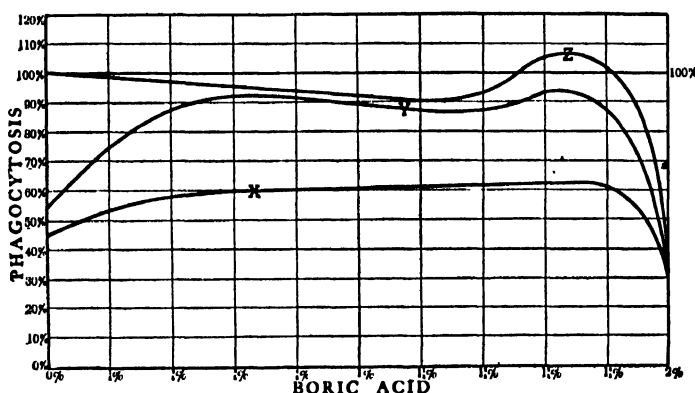


FIG. 10. Influence of boric acid on Wright's index. Curves from Fig. 9, plotted to the same scale.

the corresponding variations in Wright's index in Fig. 12. A duplicate experiment is given in Fig. 13, and the average of the two experiments in Fig. 14.

From this figure it is seen that the addition of quinine hydrochloride gives, from the first, an increase in phagocytosis, the increase reaching a maximum (20 per cent.) as soon as the concentration reaches $1/200$ per cent. A further increase in quinine hydrochloride causes a decrease in phagocytosis, phagocytosis being reduced to normal by the time the concentration reaches $1/120$ per cent., and apparently completely ceasing soon after the concentration reaches $1/40$ per cent.

Whether the observed increase is in the nature of a permanent stimulation, or is only a temporary stimulation to be succeeded by a depression, has not been determined.

Summary.—Judging from counts made at the end of fifteen, thirty and sixty minutes the following influences on phagocytosis have been demonstrated:

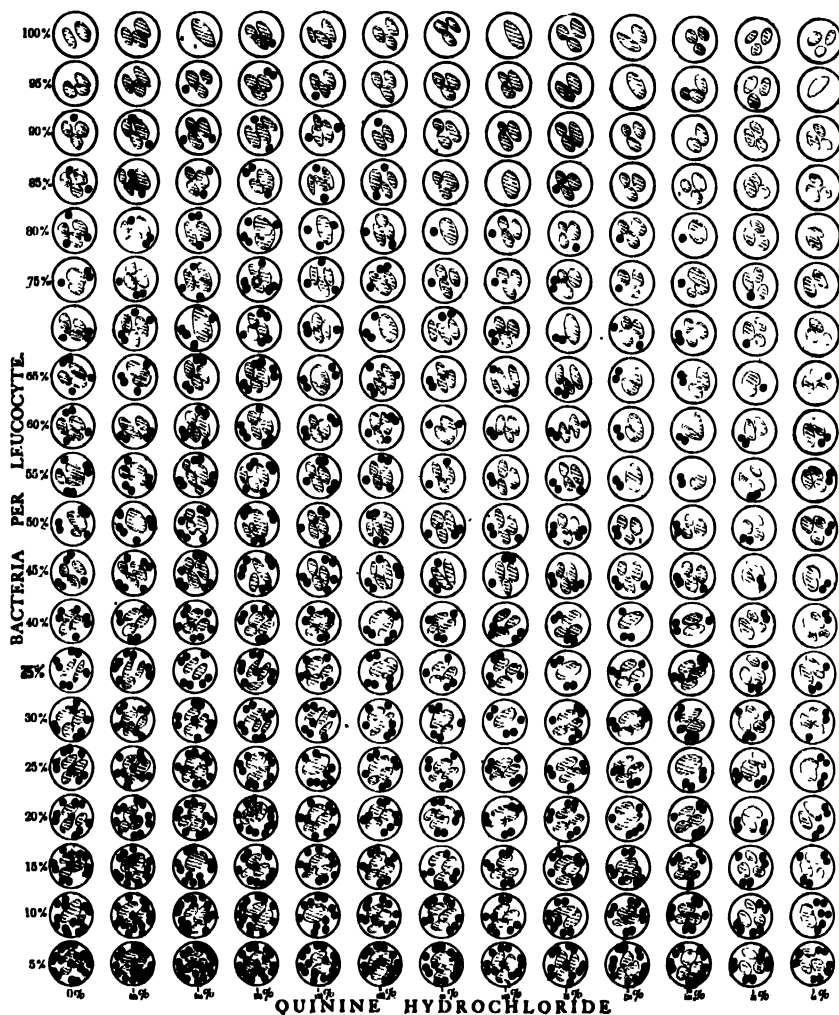


FIG. 11. Influence of quinine hydrochloride on phagocytosis. Human leucocytes; average of 15-, 30- and 60-minute counts; Experiment II.

1. Carbolic acid, added in increasing amounts to experimental tubes, causes from the first a decrease in phagocytic power, phagocytosis falling off one third by the time the concentration reaches

2/9 per cent., seven eighths by the time it reaches 1/3 per cent., and completely ceasing at 1/2 per cent.

2. Mercuric chloride, in concentrations less than 1/120 per cent.,

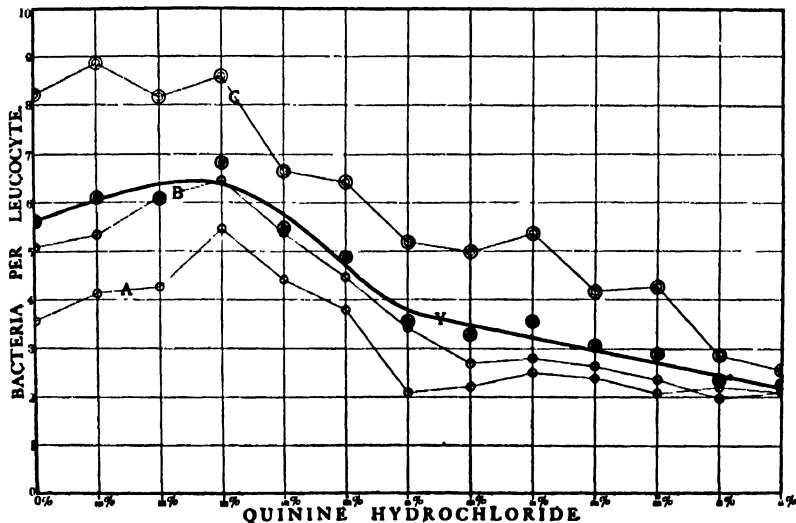


FIG. 12. Influence of quinine hydrochloride on Wright's index. Experiment II. A, B and C = 15-, 30- and 60-minute counts; Y = average.

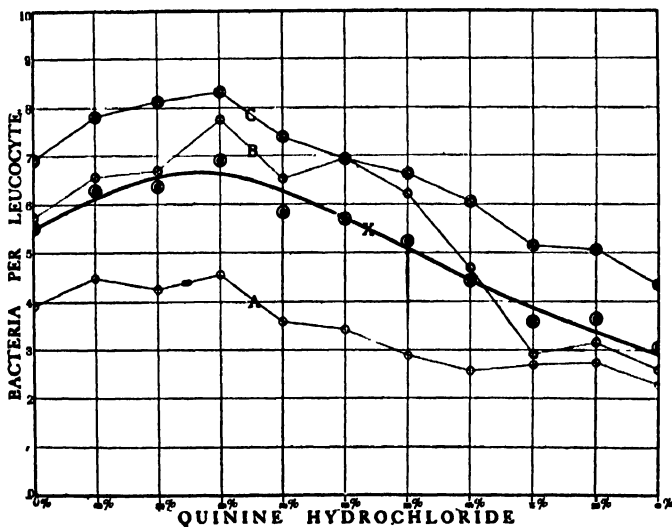


FIG. 13. Influence of quinine hydrochloride on Wright's index. Experiment I. A, B and C = 15-, 30- and 60-minute counts; X = average.

causes a transient stimulation in phagocytosis, followed by a depression. In larger amounts it causes a permanent depression from the first, phagocytosis apparently completely ceasing soon after the concentration reaches 1/60 per cent.

3. Boric acid, in concentrations less than 1½ per cent., causes a transient stimulation in phagocytosis, followed by a depression. As the concentration increases above 1½ per cent. there is a rapid fall in phagocytic power, phagocytosis apparently completely ceasing soon after the concentration reaches 2 per cent.

4. Quinine hydrochloride, added in increasing amounts, causes a

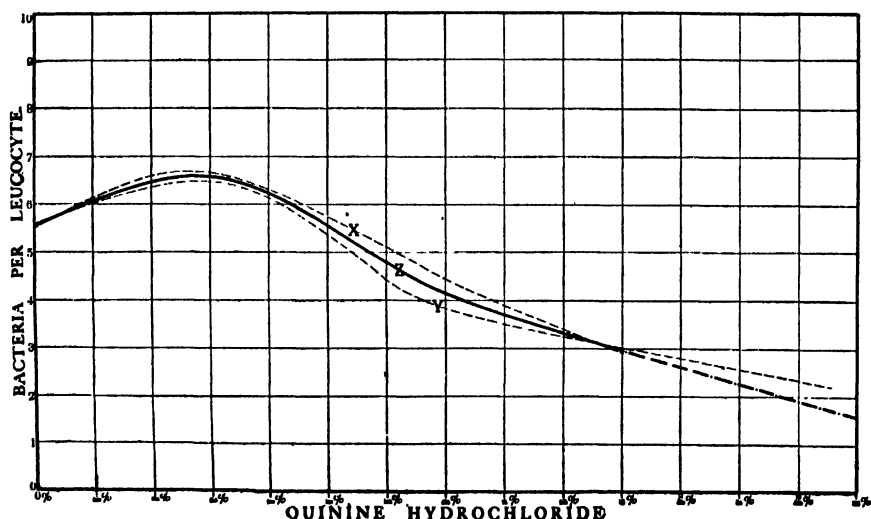


FIG. 14. Influence of quinine hydrochloride on Wright's index. Curves X and Y, from Figs. 12 and 13, plotted to the same scale; Z = average.

stimulation in phagocytosis, phagocytosis reaching a maximum as soon as the concentration reaches 1/200 per cent. A further increase in quinine hydrochloride causes a decrease in this stimulation, phagocytosis being reduced to normal as soon as the concentration reaches 1/120 per cent. In larger amounts quinine hydrochloride causes a depression in phagocytosis, phagocytosis apparently ceasing soon after the concentration reaches 1/40 per cent. Whether the observed stimulation is a permanent stimulation, or a transient stimulation to be succeeded by a depression has not been determined.

A FURTHER CONTRIBUTION TO THE KNOWLEDGE OF THE OPSONINS.¹

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(From the Laboratory of Dr. Charles E. Simon.)

In a previous communication I have drawn attention to certain fallacies which attach to Wright's method of estimating the opsonic content of the blood and have suggested that more accurate information may be obtained by diluting the blood in varying proportions and by determining the percentage of phagocytizing cells in the resulting preparations. In order to obtain comparable results I proposed the use of maximal numbers of bacteria, as a matter of routine, so that deviations from the normal, in either direction, would be the more striking. The report of the work, in which I was assisted by Drs. R. V. Lamar and Bispham, was submitted to the Rockefeller Institute on the first of March, 1906. Its publication was unfortunately much delayed and it has thus far appeared only in part.²

Since that time Wright's work has attracted widespread attention and investigators generally have been following his methods. In the further prosecution of our own work it became necessary, for purposes of comparison, to follow Wright's technique at least in part, and above all to obtain an index which should be directly comparable to Wright's index.

After certain preliminary investigations I then suggested that by comparing the percentage of phagocytizing leucocytes in the case of a specimen of blood under investigation with the figure corresponding to a specimen of pooled normal blood serum, terming the latter

¹ Conducted under a grant from the Rockefeller Institute for Medical Research. Received for publication June 20, 1907.

² Simon, C. E., and Lamar, V. R. A Method of Estimating the Opsonic Content of the Blood and Other Fluids. *Johns Hopkins Hospital Bull.*, 1906, xvii, 27. Simon, C. E., Lamar, R. V., and Bispham, W. N. A Contribution to the Study of the Opsonins. *Jour. of Exper. Med.*, 1906, viii, 651.

value 1, an index is obtained which is comparable directly to Wright's index. This index I have termed the "percentage index," as contrasted with Wright's "bacillary index." Working with *Staphylococcus aureus* emulsions prepared with special care and throwing out of the counts cells which had manifestly gotten into clumps (which after all not even with staphylococci can be altogether avoided) I could convince myself that the percentage index and the bacillary index may agree to the second decimal and that the results obtained with either method are directly comparable with each other. This fact having become established it was next ascertained that the percentage index could be used as a direct check upon the bacillary index. It was necessary, however, to work with fairly thin emulsions, viz., such as would furnish values of not much more than 50 per cent. for the normal control blood, in cases at least in which the bacillary index would suggest a material increase of the opsonic content. A normal value of 50 per cent. would allow for an increase of the patient's blood to 100 per cent., giving a maximal percentage index of 2. For most purposes this is sufficient; otherwise the blood must be progressively diluted, and the degree of phagocytosis in the diluted specimens compared with correspondingly diluted normal serum. With low values, on the other hand, the emulsions need not be so thin.

A few examples will illustrate the above points:

Example I.—The phagocytic index of the patient was 3.90 and of the normal control 4.88; the opsonic "bacillary" index was accordingly 0.79. The percentage of phagocytosis in the same patient was 60 and of the control 76 and the percentage index hence 0.78, showing the close correspondence of the two indices under favorable conditions.

Example II.—A patient's phagocytic index was 5.11 and that of the control 4.56; the opsonic bacillary index therefore was 1.09. The patient's percentage was 79 and that of the control 72, thus giving a percentage index of 1.09, showing a correspondence of the two indices to the second decimal.

While with especially good emulsions of staphylococci the two indices may thus agree very closely and at times actually coincide, deviations are frequently met with in routine work which show beyond all doubt that Wright's index, when not controlled by the percentage index, may give rise to erroneous conclusions; as a matter of fact results are at times obtained which are absolutely

absurd, and I have no doubt in the least that some of the phantastic curves which have been published are due entirely to errors of technique.

Example III.—The tuberculo-opsonic bacillary index in a case of suppurating bubo, in association with chancreoid was 1.93, which according to Wright would have suggested a tubercular process with systemic manifestations. As a matter of fact there was no evidence whatever of any tubercular lesion and the percentage index was normal, viz. 1.18.

Sometimes an increase of both indices shows that there is actually an increased degree of phagocytosis, but the occasionally enormous difference between the two demonstrates that the higher bacillary index has been in part at least owing to the fact that leucocytes had been included in the count which had gotten into clumps, and had thus artificially raised the phagocytic and therefore the opsonic index. This error could only have been avoided if in the normal control specimen a corresponding number of leucocytes had gotten into clumps of the same number of bacteria, which of course would have been purely a matter of accident. The suggestion naturally offers itself to exclude from the count cells which have manifestly gotten into clumps. The difficulty, however, would be to decide where to draw the line, which naturally would be more or less arbitrary. Then again it would be necessary to eliminate negative (empty) cells to counterbalance the discarded positive cells, and the question would arise in every case how many negative cells would have to be thrown out from the count for each positive cell, thus giving rise to further difficulties.

Example IV.—The staphylococcus percentage index in a case of sub-phrenic abscess was 1.46 and Wright's index 2.9, whereas dilution to 1:20 showed that there was no evidence of a material increase of the opsonins whatever, viz. 4 per cent., as compared with 8 per cent. on the part of the control.

It might of course be argued that since it is possible *at times* to obtain fairly homogeneous emulsions of organisms, this should be possible at all times, and that accordingly no control of Wright's index is necessary. Practically, however, this is not possible, even with staphylococci, and much less so with other organisms. It seems to me accordingly that a method with which reasonably

experienced laboratory workers cannot obtain uniform and corresponding results must either be abandoned, or it must in some measure be controlled or modified. My experience goes to show that the essential objection to Wright's method and the one which all "opsonic" workers realize is referable to the difficulty, if not the impossibility, of obtaining uniform emulsions. As I have said with staphylococci this is relatively small; with other organisms it is variable, and with the tubercle bacillus almost, if not entirely unsurmountable. Wright himself must realize this, as he has repeatedly changed his technique in this respect, and, it may be added, without announcing such changes, which after all are of fundamental importance. I have worked with extracted and non-extracted tubercle bacilli, with emulsions in 0.1 per cent. and 1.5 per cent. saline, but I have never seen an emulsion yet which was free from clumps.³

With other organisms still other difficulties enter into consideration. With the colon and typhoid bacilli, for example, there is usually such a profound degree of destruction with fragmentation and lysis of the organisms and coincident loss of reaction to stains even in normal blood, that a count of the organisms in the leucocytes must of necessity give rise to erroneous results. Even with staphylococci one meets with specimens every now and then, where the staining is very defective and may not be possible at all. I have seen cells filled with colorless "shells" of staphylococci, where a cursory examination would only have shown one or two stained organisms. Counting under such circumstances would have led to the most absurd conclusions, as is shown in the following table. The specimens were sent to me by Dr. North of the College of Physicians and Surgeons, New York, and were of the series which were being investigated by different laboratories for comparative purposes to ascertain the value of Wright's method. As normal control I used the pooled blood of four healthy individuals.

³Dr. Cole tells me that he is now working with tubercle bacilli which after cultivation on glycerine agar are killed off by exposure to sunlight, and that with such material he has obtained his best results. I have not yet had an opportunity to examine preparations made with such emulsions.

TABLE I.

Specimen.	Wright's Index.	Percentage Index.
1	.86	.51
2	1.11	.75
3	.81	.44
4	.90	.64
5	.88	.59
6	.88	.55
7	.90	.53
8	1.02	.52
9	1.02	.66
10	1.0	.59
11	.95	.58
12	1.11	.58

The correctness of my conclusions in reference to the accuracy of Wright's method will be borne out on examination by any one who will carefully compare the two indices. But I must insist that without the percentage method as a check upon the bacillary method the results obtained with the latter cannot be regarded as trustworthy.

During the past winter I had Dr. Knorr investigate this point in reference to the number of leucocytes that should be counted, and I append the results which were reached. The counts were made with emulsions of varying strength, and with normal blood serum. The results show that the percentage index fluctuates far less normally than the bacillary index. With emulsions containing from 666,000 to 2,000,000 organisms per cubic millimeter the percentage method yields fairly constant results, while with very thin emulsions it also becomes untrustworthy.

TABLE II.

A. *Emulsion of 2,000,000 cocci per c.mm.*

Cells Counted.	Wright's Index.	Percentage Index.
25	1.00	1.26
50	.97	1.00
75	.96	1.00
100	1.69	1.02
150	1.16	1.12
200	1.23	1.20
300	1.18	1.11
400	1.27	1.15

B. Emulsion of 666,666 cocci per c.mm.

25	1.85	1.18
50	1.32	1.16
75	1.03	1.00
100	1.25	1.02
150	1.30	1.02
200	1.12	.97
300	1.13	1.04
400	1.06	1.00

C. Emulsion of 222,222 cocci per c.mm.

25	1.20	.60
50	1.62	1.14
75	1.75	1.27
100	1.75	1.26
150	2.21	1.60
200	2.15	1.70
300	2.00	1.62

D. Emulsion of 74,074 cocci per c.mm.

25	0.66	1.00
50	0.50	0.62
75	0.57	0.70
100	1.14	1.26
150	1.18	1.15
200	0.95	0.96
300	1.05	1.11

As the normal variations in the opsonic index according to Wright may fluctuate between 0.80 and 1.20 it would follow, taking the entire series of examinations, as it stands, that Wright's method gave erroneous results in 50 per cent., while with the percentage method the percentage of error was 30. Throwing out the weaker emulsions we would have 37.5 per cent. of error for Wright's index and only 6.2 per cent. for the percentage index.

The data further show that even a count of one hundred or more cells is no safeguard against error, and that with the percentage index a count of fifty cells is sufficient.

The effect of the thickness of the emulsion upon the bacillary and percentage index is also shown in Table III, which at the same time illustrates how closely under favorable conditions the two will agree; it further emphasizes that the percentage index is a valuable check upon the other.

TABLE III.

No. of Cocci per c mm.	No. of Cocci per Leucocyte		Percentage of Phago- cyting Cells in		Wright's Index.	Percentage Index.
	(a) Patient.	(b) Pool.	(a) Patient.	(b) Pool.		
3,750.000	2.54	3.15	87	82	.80	.94
1,865.000	1.68	1.90	68	70	.88	.85
937.500	1.26	1.36	58	65	.90	.89
468.750	1.02	1.78	50	45	1.30	1.11
234.75	.73	.71	37	33	1.02	1.05

Working with the two indices side by side during the past winter, I found, as I have said before, that under favorable conditions the two may coincide. If an increase or decrease of Wright's index is associated with an increase or decrease of the percentage index, it may be assumed that such an increase or decrease is not due to errors of technique, and may be interpreted as actually expressing what Wright's index can express. If, on the other hand, the one goes up and the other down, experience has taught me that in such cases the percentage index should be accepted in lieu of the other.

It will be noted that I have just used the expression "what Wright's index *can* express." I do so advisedly, for I am still of the opinion elaborated in a previous paper, that Wright's method does not furnish a proper index of the quantity of opsonic material which is actually present, and I still insist that the dilution method gives results which are more in accordance with the actual facts. I have pointed out that conditions may occur in which the opsonic content of two specimens of concentrated blood serum is apparently the same, but that on progressive dilution the one rapidly loses its opsonifying power, while the other may retain it to a marked extent. This may be observed not only under pathological conditions but at times also in perfect health. I have given examples in one of my previous communications and Amberg⁴ has brought out the same point in his studies on the opsonic content of the blood of infants.

It may be objected that in my previous studies emulsions of constant bacterial content were not used, but this, I think, is invalidated by the fact that we always used maximal amounts, and that as a consequence variations from the normal are brought out into

⁴ Amberg, *Jour. of Amer. Med. Assoc.*, 1907, xlviii, 304.

bolder relief. After my second winter's work I feel as confident, as I did a year ago, that Wright's method does not furnish a proper index of the opsonic content of the blood, and I am aware of the fact that many of those who have worked upon the same problem have reached the same conclusion. I am not willing, however, to discard the principle of the matter, but would urge other opsonic workers, who stand ready to abandon Wright's opsonic index, to resume their work once more, using my method of dilution. The avowed purpose of taking the opsonic index, aside from diagnostic purposes, is the idea of having a guide to dosage and frequency of inoculation in the treatment of diseases by means of bacterial vaccines. All those who have busied themselves with this subject, and have honestly and without preconceived ideas investigated it, are no doubt ready to admit that the introduction of the bacterial vaccines marks an important epoch in the advance of rational therapeutics. The point at issue at present is the question whether Wright's opsonic index furnishes the desired guide to dosage or not. I, for one, am convinced, as the result of my own work, that in the majority of cases which Wright has suggested as suitable for vaccine treatment, and using the small doses which he has advised, at intervals of one or two weeks, such treatment can be carried out without detriment to the patient, without the use of any index. I have never seen any "negative" phases under such conditions, which could not be perfectly well explained as being due to unavoidable errors of technique. If, however, the attempt at immunization be pushed by materially increasing the frequency of the injections, or by using large doses, I feel that harm may be done and that in such cases even so coarse a guide as the index is better than none. To be sure the physical signs and the patient's general condition may furnish some indication whether the dosage has been too high, but I am a little doubtful in my mind, whether it is desirable to let matters come to such a point, where the physical signs and the general condition of the patient tell us that we have done harm, even though this be but temporary. The only "negative" phases that I have seen have occurred under such conditions, and I feel confident that the result could have been avoided had lower doses been used first and controlled by the index.

The following case will serve to illustrate this point.

A little boy of eight, the subject of recurrent epulis, was injected with 575 millions of *Staphylococcus aureus* on Nov. 20th; his index on the 13th had been .62 (bacillary) and .80 (percentage); on the 15th it was 1.4 (bacillary) and 1.0 (percentage), and on the day of the injection 1.6 (bacillary) and 1.1 (percentage). During the night of the 20th to the 21st his temperature rose to 103° , followed by a profuse sweat, and on the 27th the index had dropped to .30 (bacillary) or .45 (percentage). By December 4th the two indices had risen again to .80 and .90 respectively, and on the 11th they were 1.0 and .80. On that day an injection of 600 millions was given, which was followed by a drop to .10 and .22 respectively (see Curve I).

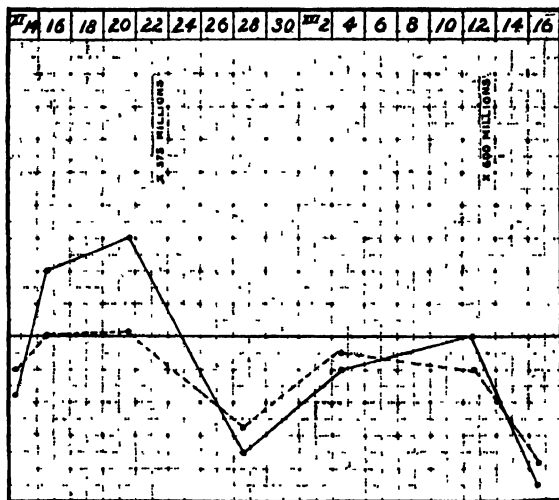


FIG. 1. Solid line indicates the bacillary index; dotted line indicates the percentage index.

Drops in the opsonic index such as these, following upon the injection of bacterial vaccines, occur very rarely in my experience, if small doses are used. They do occur, however, and I personally have the conviction, that it cannot be a matter of indifference to the patient, if his phagocytic power is practically brought to nil, and especially so in those infections in which the process of phagocytosis is one of the main stays of resistance.

During the past winter I have had occasion repeatedly to use bacterial vaccines in *acute* staphylococcus infections, and I may add, at times with very good results. The clinical course in these cases made it clear that injections given a week apart would have been without avail, and an attempt was therefore made to stimulate

the mechanism of immunization by pushing the vaccine. The question naturally was what guide to dosage should be used, and, in lieu of a better, the opsonic index was followed. These cases will be reported in detail at another occasion, but it may not be out of place to cite at least one.

The patient was a young women of about twenty-five, who a week after child-birth was seized with a chill with a temperature elevation to 104.1° ; the next day she had another chill with a temperature of 104° . From this day her temperature varied between 101° and 105° . I saw her for the first time on the evening of Jan. 21st, eight days after the onset of the fever. At that time the temperature was 104.8° ; she was quite somnolent and made the impression of a very sick woman. The physician in attendance had isolated *Staphylococcus aureus*, and she was accordingly injected with aureus vaccine. One thousand millions were given on the evening of the 21st and another dose the following morning. Her temperature promptly came to normal and remained so until the 26th. On that day and the following there was an evening rise to 102.5° . She received 750 million cocci on the evening of the 27th, and the same dose on the morning of the 28th. Her temperature promptly again came to normal and remained so. Her index (percentage) at the time of the first injection was .95; the next morning 1.0 (no negative phase) and on the 23d, viz. 36 hours after the first and 24 hours after the second injection, 1.90. The further course is seen in the curve (Curve II).

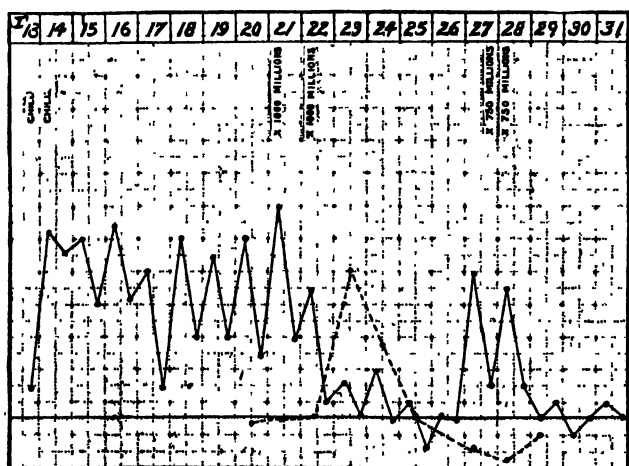


FIG. 2. Solid line indicates highest and lowest temperatures of the day; dotted line is the opsonic curve.

I have been surprised to see to what extent the injection of the staphylococcus vaccines can be carried in some cases, and manifestly not to the detriment of the patient. In one of my acute

cases 1,000,000,000,000 were injected within a week, and I may add without the appearance of a negative phase, and with an excellent effect upon the patient's condition (staphylococcus septicæmia with multiple abscesses). Nevertheless I hardly feel willing to carry the injections to such a point without some guide, better than the physical signs and the patient's general condition, and I feel convinced that the opsonic index in such cases is of service. But I repeat once more *with small doses*, such as Wright recommends, *administered at intervals of a week or two in chronic, well localized infections the opsonic index is of little, if any real value, either as a guide to dosage or to the time and frequency of inoculation.*

THE SPECIFICITY OF THE OPSONINS.

As regards the specificity of the opsonins my earlier investigations led to the conclusion that under normal conditions at least a specificity of the opsonins does not exist,⁵ and I suggested then that it is quite conceivable that as a result of infection, viz., immunization substances may be produced either *de novo*, or increased in amount, if preëxistent, which may so influence a given organism that under the subsequent action of a common non-specific opsonin, it is taken up in larger or smaller numbers, as the case may be.

During the past winter I have repeated many of the experiments of the previous year, to eliminate possible errors of technique, but have been forced to the same conclusion, viz., that *the opsonins of normal blood serum are non-specific.*

This conclusion is based upon absorption experiments which were analogous to those previously reported. Since some of the organisms, however, and notably when relatively avirulent, are taken up by the leucocytes already in physiological salt solution (*Bacillus subtilis*, *Bacillus pyocyaneus*, *Bacillus coli*), and since some of Bulloch and Western's⁶ contrary results may possibly be explained upon this basis, I chose only such organisms which had recently been isolated from infected individuals. Working with these I could find no evidence whatever that absorption of the opsonins for one organism leaves opsonins for other organisms behind. To be

⁵ *Jour. of Exper. Med.* 1906, viii, 651.

⁶ Bulloch, W. and Western, G. J., *Proc. Royal Soc.*, 1906, lxxvii, 531.

sure I never carried the absorption to a point where opsonic action ceased altogether, but in all cases there was a marked drop for both.

Example I.—About 0.5 c.c. of normal blood serum was inoculated with one-half of a twenty-four hour staphylococcus agar culture, and incubated for twenty minutes at 37° C. The percentage of phagocytosis before inoculation was 100 for the staphylococcus and 90 for the colon bacillus. After incubation the serum was centrifugalized at high speed for one hour, the supernatant fluid transferred to a new tube and centrifugalized for an additional half hour. The percentage of phagocytosis, for the same emulsions, was then 60 for the staphylococcus and 50 for the colon bacillus.

Example II.—In another experiment the values before absorption were 64 for staphylococcus and 96 for colon, and 44 and 40 respectively after absorption with the staphylococcus.

Example III.—In this instance the staphylococcus value before inoculation with staphylococci was 96 and the colon value 64, while at the end of the experiment they were 16 and 20 respectively.

While my results thus do not agree with those of Bulloch and Western it is possible, as I have pointed out, that the phagocytosis which they noted for the non-absorbing organism after absorption with the other, may have been due to non-virulence of the bacteria used and consequent spontaneous phagocytosis. In one class of his experiments Bulloch uses *Bacillus pyocyaneus* after absorption of the serum with staphylococci, and finds that the serum has lost its opsonic power for the staphylococcus, while that for the pyocyaneus is preserved. This is not at all surprising since laboratory cultures of the pyocyaneus are taken up very readily in physiological salt solution.

I have not used the tubercle bacillus in my experiments, as living virulent organisms were not available.

Bulloch's observations that inoculation of a human being with tuberculin or with staphylococcus vaccine causes a quantitative increase in the tuberculo- and staphylococcus opsonins respectively, while the staphylococcus and tubercle opsonins in turn remain unaltered, are not well adapted to either prove or disprove the point at issue. As a result of the injection of bacterial vaccines the entire immunization mechanism of the body is thrown into activity and immune opsonins possibly formed at the same time. So long as the identity of normal and "immune" opsonins, however, has not been proven it seems to me that conclusions based upon the for-

mation of the latter are not applicable in the discussion of the specificity of the opsonins of normal serum. I should like to remark nevertheless that the injection of either tubercle or staphylococcus vaccine in the small doses recommended by Wright, does not necessarily cause an increase in the opsonic index in reference either to the one organism or the other.

Example I.—Mr. C. medical student. Staphylococcus index on Jan. 29th was .90. He was injected with 750 millions of staphylococci on Jan. 30th and on Dec. 2d the index was 1.15.

Example II.—Mr. R. Staphylococcus index on Jan. 10th .95; injection of 700 million staphylococci; index on the 12th 1.04.

Example III.—Miss R. Tubercle index on Jan. 29th .83; injection of 1/1000 mgrm. of tuberculin on the 30th; index on Feb. 2d .90.

These observations thus do not tend to bear out the correctness of Bulloch's view that the opsonins of normal serum are specific and my experiments on the blood of different animals, not only of different species but of the different classes of vertebrates in general, in all of which I could demonstrate the presence of opsonins for various organisms, similarly point to the same conclusion. Particularly striking is the fact that in the lower classes of vertebrates I could demonstrate the presence of opsonins for organisms with which infection does not occur in these animals. I recall the marked opsonifying power of the blood of the terrapin, of the frog and of fowls for *Staphylococcus citreus*, as an example.

Passing from the consideration of the specificity of the opsonins in normal blood to that of the blood in the infected individual there is evidence to show that here actual specificity may exist, but I must add, does not necessarily exist. While under normal conditions the opsonic content of the blood varies within relatively narrow limits, the variations in infections are much wider. These limits, so far as the "index" goes, Wright and his coworkers have placed at 0.8 and 1.2. My inclination would be to extend them somewhat more, for I have found indices as low as 0.70 and as high as 1.25 in individuals who showed no evidence whatever of any infection. At the same time I must admit that this is exceptional. This question is an important one in deciding from how many individuals blood should be taken to make up a normal control serum. I had Dr. Knorr investigate this point with the result

shown in the accompanying table. Dr. Knorr's blood served as patient's blood and was controlled by the pooled blood of an increasing number of supposedly normal individuals. Dr. K.'s phagocytic index for staphylococcus emulsion was 1.14; the percentage of phagocytosis was 56.

TABLE IV.

No. of Persons Furnishing Control Blood.	Per Cent. of Phagocytizing Cells.	Phagocytic Index.	Bacillary Index.	Percentage Index.
1	62	1.24	0.91	0.90
2	57	1.18	0.96	0.98
3	60	1.42	0.80	0.93
4	56	1.16	0.98	1.00
5	64	1.19	0.95	0.87
6	71	1.52	0.75	0.79
7	58	1.11	1.00	0.96
8	60	1.40	0.81	0.93
9	68	1.48	0.77	0.82
10	71	1.52	0.75	0.79

From this table it is clear that it is usually not necessary to make up a pooled serum from many individuals, and for routine work the serum from one normal person is really sufficient, providing that this has been previously and repeatedly tested to exclude the possibility of its being normally either unusually high or unusually low. The serum of women during the menstrual period cannot be used as control serum, as the index during that time is abnormally low (see Table V).

TABLE V.

	Percentage Index.
A	0.57
B	0.54
C	0.53
D	0.24
E	0.72
F	0.54

As the lowered index does not affect one single species of organisms, but seems to be general, this fact also might be adduced as further supporting the view of the non-specificity of the normal opsonins at least. It is quite conceivable, considering the more or less uniform occurrence of constitutional disturbance at that time, that substances may be circulating in the blood which produce an inhibitory effect upon the opsonins.

While then the opsonic index in normal individuals (barring women during the menstrual period, and the exceptional individuals of whom I have spoken) varies within comparatively narrow limits, most remarkable deviations from the normal figure may be met with in disease. In their earlier work Wright and Douglas⁷ reported a series of staphylococcus infections (furunculosis, syccosis, acne, etc.) in all of which the opsonic index was abnormally low, ranging from 0.1 to 0.87. They add that they have not come across any instance of the association of a normal phagocytic power with a staphylococcus infection. Analogous results were obtained in cases of tuberculosis. In one series of seventeen cases the values ranged between 0.4 and 0.85, including cases of tubercular peritonitis, laryngeal phthisis, psoas abscess, lupus, pulmonary phthisis, etc. In a series of twenty-five cases of pulmonary tuberculosis other than the acute form, Lawson and Stewart⁸ also found low figures, on the whole, although in some the values were but little, if at all, below the normal average, viz., 0.5 to 1.0.

Subsequently Wright pointed out that low values are the rule in strictly localized infections, while in systemic infections abnormally high values may be observed.

As the index in the various infections shows deviations from the normal which usually only affect the offending organism, and as the injection of bacterial vaccines in such cases has a direct effect upon the corresponding bacterial index, Wright and his collaborators conclude that these facts indicate that the opsonins in the infected organism are specific. As they do not admit that a material difference exists between the opsonins of normal serum and the serum of infected individuals they further conclude that the normal opsonins also are specific. I have pointed out that there is no satisfactory evidence to support this view.

In studying the question of the specificity of the opsonins in the infected organism, which we shall refer to for the present, as a matter of convenience, as "immune opsonins," a series of absorption experiments were undertaken which were perfectly analogous to those described before, but in which the serum of infected indi-

⁷ Wright and Douglas, *Proc. Royal Soc.*, 1904-5, lxxiv, 147.

⁸ Lawson and Stewart, *Lancet*, 1905, ii, 1679.

viduals was exhausted, viz., of persons and animals which had been treated with bacterial vaccines. My experiments thus far have reference largely to staphylococcus and typhoid infections, but in neither could I find any evidence that absorption with one organism removed a homologous opsonin and left a heterologous opsonin behind. Working with dried and pulverized tubercle bacilli, on the one hand, and staphylococci, on the other, I have obtained results which corresponded in a way to those of Bulloch, viz., after absorption with staphylococci I occasionally found a slightly higher value for the tubercle bacillus than for the staphylococcus. If the experiment was reversed, however, the tubercle value was still higher. This leads me to think that the causes of the higher retention lay in the powdered bacilli and not in the serum, and that a different result might have been reached, if live virulent tubercle bacilli had been used instead. But, as I have said, such were not available at the time. As far as they go, however, my absorption experiments do not bear out the existence of specific "immune" opsonins.

A further argument which has been advanced as supporting the idea of the existence of specific opsonins, has reference to the greater thermolability of the normal opsonins as contrasted with the thermolability of the opsonins of "immune" sera. Wright, to be sure, does not admit that a material difference exists between the two, for he says:⁹ "Manifestly the plain teaching of our experiments is, that the opsonin which is found in the heated immune serum of a patient who has responded to tubercular infection (I assume that his remarks would apply to any other infection in which phagocytosis plays a *rôle*), or as the case may be to the inoculation of a tubercle vaccine, does not differ, with respect to its resistance to heat and sunlight, from the opsonin which is found in the unheated normal serum. A precisely similar conclusion with respect to the identity of the opsonins found respectively in unheated normal and heated immune sera. . . ."

Other observers do not agree with Wright in his assumption that the opsonins of normal and immune sera are identical, notwithstanding his attempted explanation to this end. He himself, how-

⁹ *Proc. Royal Soc.*, 1906, lxxvii, 224.

ever, manifestly attaches special significance to the fact that the opsonins of immune sera may be thermostable, or, as it would possibly be better to say, that they contain a thermostable component. The fact that he suggests that the thermostability of the opsonin toward a single organism may be used for diagnostic purposes shows that in his mind this thermostability is intimately associated with the question of specificity. In two tables he gives examples illustrating the thermostability of the opsonins toward the tubercle bacillus and introduces them with the remark that they represent a typical selection from a very extensive body of observations which furnishes the basis for the preceding statement as follows: "When a serum is found to retain in any considerable measure, after it has been heated to 60° for ten minutes, its power of inciting phagocytosis, we may conclude that 'incitor elements' have been elaborated in the organism, either in response to auto-inoculations occurring spontaneously in the course of tubercular infection, or as the case may be, under the artificial stimulus supplied by the inoculation of tubercle vaccine." This is important, as Wright himself thus restricts the drawing of any diagnostic inferences from absence of thermostability. It is remarkable, however, on the one hand, that many cases do occur in whom there is every evidence of systemic infection, without thermostability of the opsonins, and that thermostable opsonins, on the other hand, may be found in persons in whom there is no evidence of infection.

TABLE VI.

Showing a series of cases in which active phagocytosis was not obtained after heating the sera for 10 minutes to 60° C.

Number.	Nature of Infection.	Unheated Bacillary Index.	Serum Percentage Index.	Heated Bacil. Index.	Serum Percent. Index.
Miss R.	Vesical tuberculosis.	.57	.66	0	0
Mr. G.	Tuberculosis of cæcum.	1.10	.91	.08	.03
Mr. G.	Tuberculosis of cæcum.	.95	1.08	.17	.25
Mr. B.	Renal tuberculosis.	.75	.80	0	0
Miss M.	Tubercular conjunctivitis.	—	.81	—	.09
R.	Staphylococcus suppuration.	.53	.94	.03	.20
Miss Sch.	Staphylococcus septicæmia.	.08	.27	.03	.11
Miss Sch.	Staphylococcus septicæmia.	2.36	1.53	.08	.26
G.	Abscess of finger (staph.)	1.63	1.26	.06	.06
Z.	Drained pelvic abscess (staph.)	1.36	1.20	.06	.06
M. D.	Tubercular hipjoint.	1.2	.9	0	0
X.	Tubercular elbow joint.	—	.85	—	0

Of cases in which marked thermostability was observed without any evidence of infection of any kind I may mention two. In the first there was from time to time hæmaturia of slight grade without any apparent cause. The patient was examined in great detail by Dr. H. A. Kelly, with negative result; tubercle bacilli could not be demonstrated in the urine; an injected guinea-pig remained well and the tuberculin test was negative. The tuberculo-opsonic index on three successive days varied between .52 and 1.20; corresponding to the latter value the index with the heated serum was .39.

In the second case there was likewise no evidence of tuberculosis with the usual methods of examination; the tuberculo-opsonic index, however, varied between .36 and 1.6 with the unheated serum and between .26 and .45 with the heated.

In studying these negative cases and contrasting them with my positive cases and with those of Wright, I cannot help but feel that in some of the positive cases the reaction in question is specific, but that in the negative cases we are dealing with normal non-specific opsonins. The difficulty may lie in our present inability to differentiate between infection *per se* without immunity production and infection associated with immunity production. This is even more so the case when we study the opsonic index toward a given micro-organism from the standpoint of differential diagnosis, aside from the question of thermostability. Wright's conclusions that deviations from the normal values may be interpreted as evidence of infection with the homologous organisms suggest very strongly that a specificity exists, but it is difficult to understand, on the other hand, why so many cases of infection do not show any material deviation from the normal and cases, moreover, in whom there could be no doubt that systemic reaction was going on. Table VII shows a series of such cases.

In one case of acute staphylococcus septicæmia which ended fatally on December 15 and in which daily observations had been made since December 8, an abnormal index was only noted on two occasions, viz., .40 on December 8 and 2.0 on the twelfth; on the remaining days the values were entirely normal.

While my observations bear out the correctness of Wright's conclusions in a general way, that deviations from the normal for a

TABLE VII.

Showing a series of cases of both localized and systemic infections in which no material deviation from the normal-homologous index was obtained.

Name.	Disease	Percentage Index.
Miss W.	Acne	1.0
Mr. B.	Renal tuberculosis	1.25
Mr. G.	Tuberculosis of the cæcum	1.25
L. B.	Glandular tuberculosis undergoing treatment with with tubercle vaccine90
S.	Tubercular hipjoint	1.10
J.	Tubercular hipjoint87
C.	Suppuration following operation	1.00
Dr. S.	Furunculosis75
Sp.	Suppuration following burns93
R.	Suppuration following operation94
W.	Staphylococcus abscess of neck88
J.	Suppurating bubo81
W.	Tubercular adenitis	1.18
F.	Staphylococcus septicæmia (multiple abscesses)90
L.	Staphylococcus septicæmia95

given organism may be regarded as important evidence from the standpoint of differential diagnosis, normal values can manifestly not be interpreted as excluding the possibility of infection with the organism under consideration.

But, as I have just said, my conclusion in reference to deviations from the normal coincide with those of Wright, only in a general way, for I have seen marked deviations both upward and downward for various organisms in cases in which there was not a corresponding infection; and the fact, moreover, that in some of these cases, at any rate, the deviations had reference not to a single species of organism seems to me to be further evidence to show that the opsonins in question were not specific.

One case in point (Mason) was one of syphilis of the liver with ascites; there was no evidence whatever of tuberculosis, while the tuberculo-opsonic index was .27.

In a second case of hepatic syphilis with ascites both the tuberculo-opsonic index and the staphylococcus index were increased, 1.4 in the case of the former and 1.3 in the latter (the bacillary indices were 2.6 and 2.3 respectively).

In a case of sarcoma of the breast and the axillary glands the tubercle index was .40 and the staphylococcus index .73 (the bacillary indices .39 and .37).

A typhoid patient in the second week of the disease gave a staphylococcus index of 1.46 (1.85 bacillary), in the absence of any apparent staphylococcus infection.

I further append some staphylococcus citreus values, obtained with my method of dilution, in cases in which a citreus infection could hardly be assumed to have existed.

TABLE VIII.

Disease.	1:20 Dilution.	1:30 Dilution.	1:40 Dilution.
Normal (average).	37.2	19.4	9.2
Appendicitis. ¹⁰	96	88	—
Myelogenous leukæmia. ¹¹	—	6	0
Syphilitic ulcer of cæcum.	18	0	0
Carcinoma (generalized).	0	3	0
Appendicitis.	96	92	80
Retroperitoneal sarcoma.	—	68	64
Syphilitic cirrhosis.	100	96	—
Pneumonia.	100	92	88
Pneumonia.	100	88	84
General carcinomatosis.	100	92	64
Appendicitis.	100	100	100
Pneumonia.	100	88	60
Appendicitis.	20	—	0
Beri-beri.	4	0	0
Ruptured extra-uterine pregnancy.	12	4	0
Melanotic sarcoma.	8	—	0
Pernicious anæmia.	92	42	28
Spleno-medullary pseudo-leukæmia.	88	60	20

With these various facts before us, I believe, the conclusion is justifiable that *granting the opsonins to be definite entities their specificity, even in cases of infection has not yet been established, although certain data seem to point in that direction.*

OPSONIC IMMUNITY.

From the writings of Wright the majority of his readers no doubt have gained the impression that as a consequence of successive inoculations of bacterial vaccines an opsonic immunity gradually becomes established, which would be characterized by a persistently high index, a continuous "high tide phase" to use Wright's own expression. Unfortunately there are no exact data published by Wright to throw light upon this question. My own investiga-

¹⁰ I have included appendicitis cases in this table, since staphylococci are only very rarely found under these conditions and there is little reason to assume that they were staphylococcus infections.

¹¹ This low value was obtained during a period of great improvement in the patient's condition with practically normal blood picture.

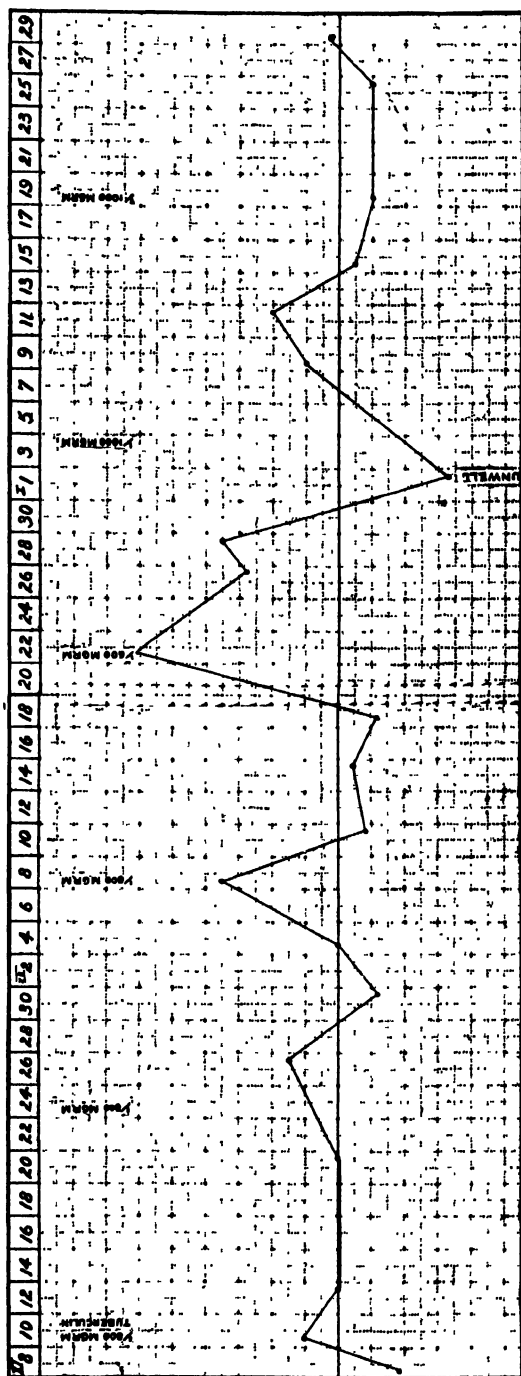


Fig. 4. Mrs. N. Vesical tuberculosis; under treatment nearly a year with great improvement.

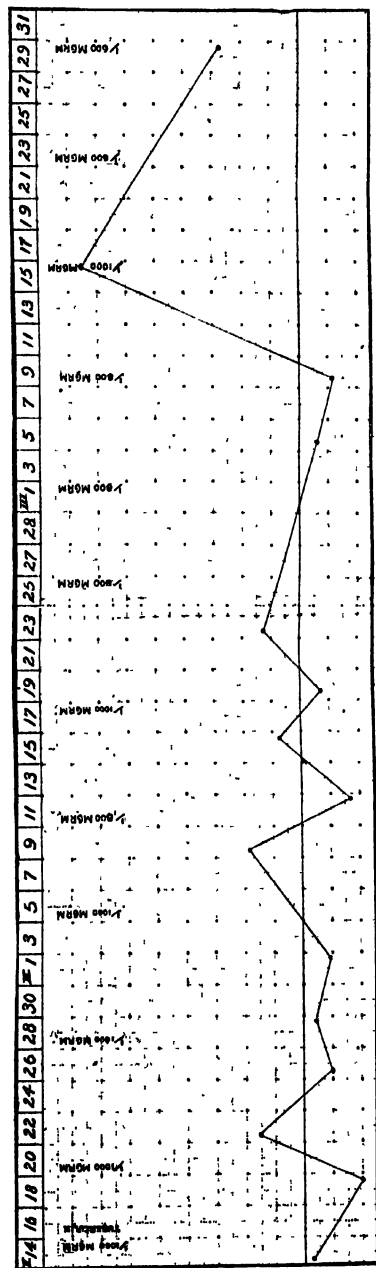


FIG. 5. Mr. B. Tubercular horse-shoe kidney and calculous pyelitis; gain in weight under treatment, but tubercle bacilli continue in the urine.

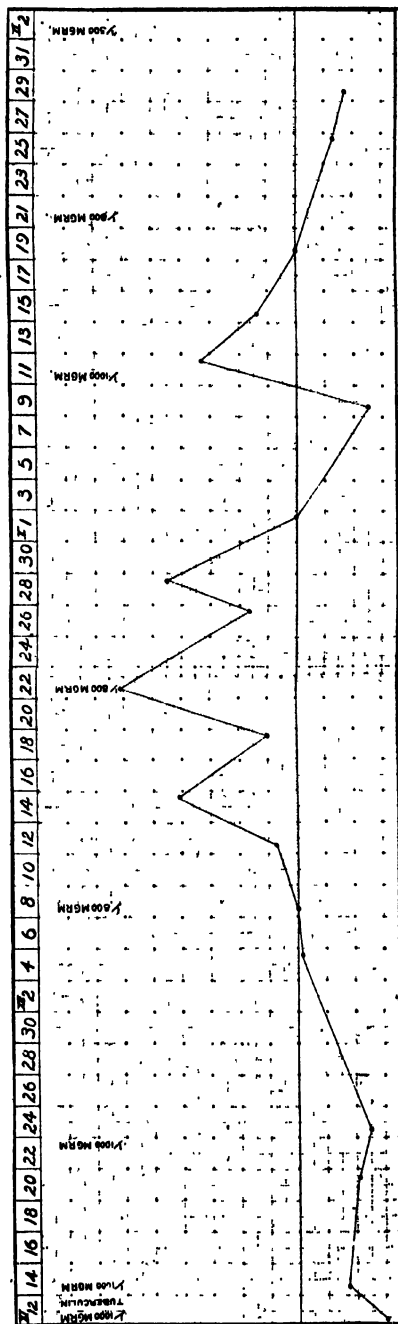


FIG. 6. Miss H. Tubercular peritonitis; operation; tuberculin treatment; recovery after severe illness.

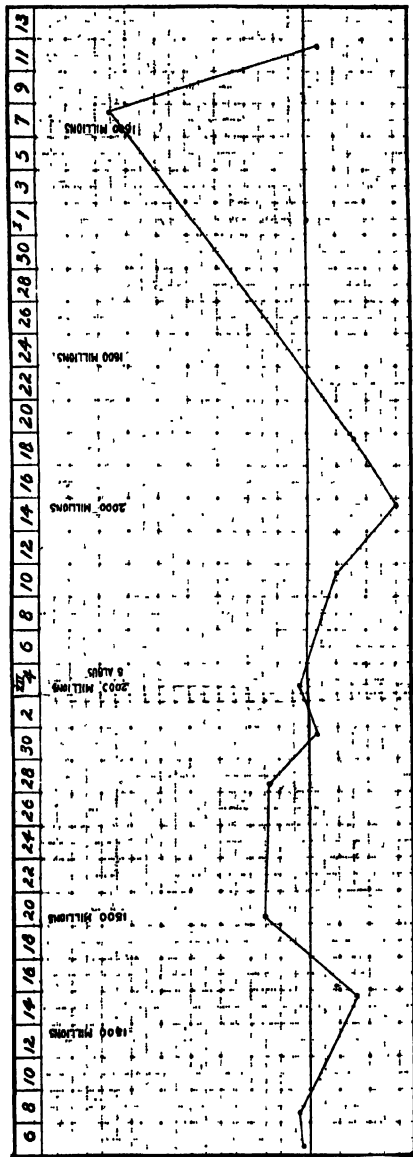


FIG. 7. Mr. A. Acne; recovery.

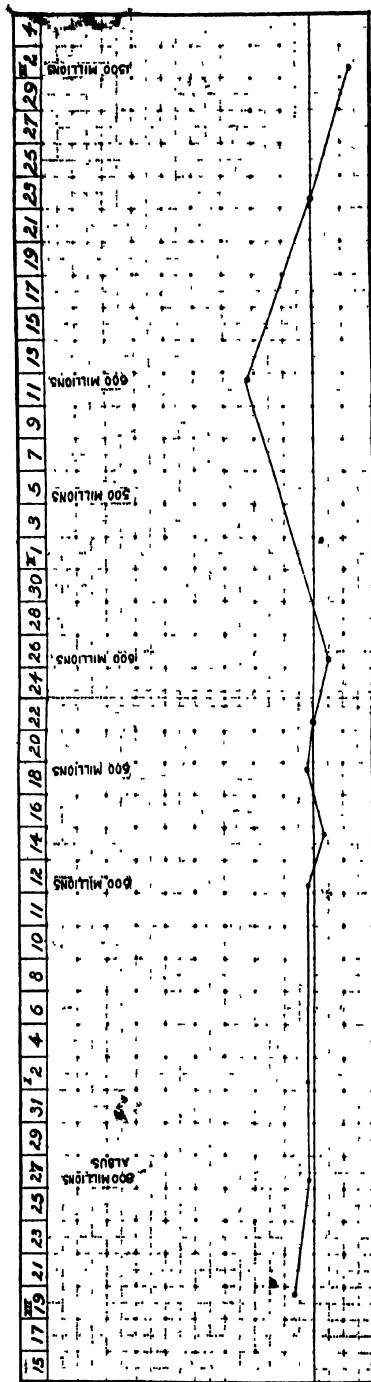


FIG. 8. Miss F. Acne; recovery.

tions in this direction have reference almost exclusively to tubercular and staphylococcus cases. I have already pointed out that many cases, of this kind, occur in which the index at the very outset of immunization is not materially lowered and that no very marked fluctuations may occur. In such cases successive inoculations of the corresponding vaccines brought about no material change in the height of the index, as compared with the normal. With small doses no negative phases were observed which could not readily be explained as being due to unavoidable errors of technique. Within a day or two there was usually (but not always) a rise in the index which then dropped again to near the normal line (Curve III, IV and V). A distinct and persistent rise was only observed in cases in which the nutrition of the patient was markedly below the normal at the start. As this improved (during the process of immunization) the index gradually rose to normal, without any marked fluctuations. In one instance (Curve VI) remarkable variations then occurred (the patient steadily improving), but it will be noted without a persisting upward tendency.

In no instance was a continued high tide observed and after several months of active immunization the index was not found higher than normal (Curve VII and VIII). The patient from whom Curve IV was taken had been under vaccine treatment for nearly a year.

Animal experiments led to the same result. We may accordingly conclude that in the case of the tubercle bacillus and the staphylococcus a study of the opsonic content of the blood by Wright's method shows no evidence of the development of an opsonic immunity, which likewise argues against the specificity of the opsonins. The available literature having reference to this point is still too meager to furnish any data which could be interpreted as evidence either for or against this conclusion, but I note that Bulloch states that "in most cases the high tide is in the course of a few days succeeded by a fall again. . . . In spite of this the improvement may continue, a fact which leads me to assume that there are other factors at work which at the present time cannot be measured." This coincides with my own views and I would emphasize that no matter what the future of the opsonic "index"

may be there can be no doubt whatsoever that remarkably beneficial results can be reached by means of bacterial vaccines and that Wright deserves full credit for having generalized this principle of immunization in human therapeutics.

OPSONINS AND AGGRESSINS.

In order to ascertain whether the lowered opsonic content which may be observed in the various bacterial infections is referable to an insufficient production of opsonic material or to the simultaneous presence of inhibitory substances (aggressins, endotoxins), a series of experiments was undertaken in which the blood of infected individuals was added to normal blood and the phagocytic power of the mixed blood compared with the normal. My results are not yet numerous enough to warrant any definite conclusions, but they show that in some instances in which the patient's index was low, a distinct inhibitory effect occurs. That this is not the expression of dilution merely, with a serum of lowered bacterial content, is clear from the fact that the substitution of saline solution for the patient's blood does not cause a corresponding effect.

Example I.—The patient was a young girl with generalized septicæmia following an appendectomy. Her opsonic content on Nov. 25, measured by my percentage method, was 18; the normal control value was 72, and her percentage index accordingly .25. A mixture of equal volumes of the control serum and saline solution gave 32 per cent., corresponding to an index of .44, while a mixture of the patient's and the control serum gave only 16 (index .22). There was thus a drop of 50 per cent., which, I think, cannot be otherwise interpreted than as being due to the action of inhibitory substances in the patient's serum.

Such results, however, were exceptional and not constant even in one and the same individual. At times, indeed, the mixed sera gave higher values than the control, conveying the impression as though the normal serum had liberated a bound quatum of the patient's opsonins. If this idea of bound opsonins in the infected individual could be shown to hold good, it would explain the curious fact that notwithstanding the existence of such infections in which the process of phagocytosis is the only method of defense of which we have evidence, normal values are so frequently observed although active symptoms exist, from which we would expect to find the opsonins either high or low, but certainly not normal.

Example II.—On the sixteenth of February the percentage value of the patient referred to in Example I was 20, while the control was 72; her index hence was .27. The mixture of control and saline gave 40, *i. e.*, an index of .55, while that of the patient's and the control serum was 80, corresponding to an index of 1.11 and representing an increase of 100 per cent.

Example III.—In a woman following hysterorrhaphy a mixed staphylococcus and streptococcus septicæmia developed. Her percentage value for the staphylococcus was 76, while the control was exactly the same and her index hence 1.00. The mixture of the control and saline gave 60 (index .78), while that of the patient's serum and the control was 92 and the index hence 1.21, representing an increase of about 50 per cent.

Working with exudates I have repeatedly noted that their addition to normal serum causes a more marked drop in the phagocytic index than the corresponding blood serum. This is manifestly not due to a lower content in opsonic material *per se*, as transudates and cystic fluids likewise furnish lower values; but their admixture (when fresh) to normal serum does not bring about a corresponding decrease.

Example IV.—The percentage phagocytic value of a specimen of fresh hydrocele fluid was 12, while that of the normal blood serum was 72; the index thus was .16. The mixture of control serum and saline gave 52 (index .72) and that of the serum and hydrocele fluid 48, corresponding to an index of .66, thus showing no material effect.

Example V.—A specimen of fluid was obtained from an intraligamentary cyst; its phagocytic value was 16 per cent. and that of the control serum 76; the index hence .21. Equal parts of serum and saline gave 42 per cent. (index .55) and of saline and cystic fluid 48 per cent. (index .63).

Example VI.—Fluid from an ovarian cyst; phagocytic value 4 per cent. (index .10); equal parts of control serum and saline gave 32 per cent. (index .80) and of saline and cystic fluid 28 per cent. (index .90).

There is thus no evidence of any inhibitory action whatever. But with exudates, as I have said, there is often a marked effect upon the phagocytic power which I hardly think can be interpreted otherwise than as being due to definite inhibitory substances.

Example VII.—Mrs. C., the patient referred to before (Example III). The staphylococcus value of the pleural exudate in this case was 48 (index .63), while with the streptococcus no phagocytosis whatever was observed. It is interesting to note that the exudate contained streptococci in large numbers, which were cultivated from the exudate and from the patient's blood, while the staphylococcus infection was apparently localized. The mixture of normal serum and saline for the staphylococcus gave 76 and for serum and exudate 80 (index 1.05), while with the streptococcus the values were 42 and 12 respectively, giving an index of .23, thus showing a decrease of over 70 per cent. (!), which, as I have said before, must be attributed to the action of inhibitory substances.

The following example shows a similar effect.

Example VIII.—The patient was an old gentleman, aet. about 70, with empyema. The phagocytic power of his blood serum was 88 per cent., corresponding to a control of 72, thus giving an index of 1.11; the value of the exudate was 12 and the index hence .16. Equal parts of normal serum and saline gave 60 per cent. (index .88) and of normal serum and exudate 12 per cent. (index .16) showing a drop of about 80 per cent.!

The patient died a few days later and the exudate which was drawn off after death again examined. Its direct phagocytic value was 4 (index .10); normal serum and saline gave 32 (index .80) and saline and exudate 8 (index .20), thus showing a decrease of 75 per cent.!

Example IX.—(Same patient as Example I.) Within forty-eight hours preceding death ascites developed and in the exudate chain cocci, diplococci and bacilli were found in immense numbers. Some of the material was freed from bacteria by prolonged centrifugation and then showed a staphylococcus value of 4 per cent. (index .16); normal serum and saline gave 16 per cent. and with serum and exudate no phagocytosis whatever occurred—a loss of 100 per cent.!!

Of the character of the inhibitory substances in the blood and in the exudates of infected individuals nothing is known. I have some evidence, which suggests that they are comparatively unstable, but am not prepared to discuss this phase of the subject at the present time. Whether or not they are of bacterial origin and of the nature of aggressins (viz., endotoxins), or whether they are products of autolysis referable to the cells of the infected individual future research will have to show, but their demonstration in the blood and exudates renders it possible, if not probable, that the low opsonic values which are so frequently seen in the various infections may be due in part at least to the simultaneous presence of antagonistic substances.

CONCLUSIONS.

1. The determination of Wright's index of the opsonic content of the blood and other fluids of the body is open to serious and in part unavoidable errors and should be abandoned in its present form.

2. Conclusions based upon the determination of the opsonic content of the blood, according to Wright's method, are accordingly not uniformly reliable.

3. The percentage index is a valuable check on Wright's bacillary index, but likewise does not furnish an adequate idea of the opsonic content of the blood, unless carried out with progressive dilutions to the point of opsonic extinction.
4. The opsonins of normal blood serum are not specific.
5. The specificity of the opsonins in "immune" sera has not been satisfactorily established, but appears probable.
6. An opsonic immunity, in the sense of a continued high opsonic content of the blood does not exist.
7. In the blood and exudates of infected individuals substances may be present which exercise an inhibitory effect upon phagocytosis.

OPSONINS OF INFLAMMATORY EXUDATES.¹

By EUGENE L. OPIE.

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There is much uncertainty concerning the relationship of so-called opsonins to other bodies concerned in the production of immunity and available data is insufficient to indicate with what accuracy the opsonic power of the serum measures resistance to bacteria either in normal or in immunized individuals. Wright has found the opsonic power of the serum with certain bacterial infections much below the normal and believes that a low opsonic index indicates susceptibility to the particular microörganism which has been employed in testing the opsonic activity of the blood serum. The low opsonic index observable with certain pyogenic and tuberculous infections is believed by Wright to antedate infection, being indeed its cause. There can be no doubt that the opsonic power of the blood is subject to considerable variation, but non-bacterial factors which depress it are but little known. Hektoen and Ruediger² have shown that a variety of inorganic salts inhibit the opsonic activity of the serum in the test-tube, but what effect similar substances have during life is not known.

Wright and Reid³ further have shown that exudates produced by a given bacterium may exhibit partial or complete absence of opsonin for this microörganism. The fluid in the peritoneal cavity with tuberculous peritonitis may contain no opsonin for tubercle bacillus; it has, to use the expression of Wright and Reid, a low bacteriotropic tension.

The following experiment shows that an inflammatory exudate caused by one microörganism may contain no opsonin for this or for other microörganisms. A fatal quantity of *Staphylococcus pyogenes aureus* has been injected into the peritoneal cavity of the

¹ Received for Publication July 2, 1907.

² *Jour. of Infec. Diseases*, 1905, ii, 128.

³ *Proc. of the Roy. Soc.*, 1906, lxxvii, 194.

guinea-pig and the power of the serum to opsonize *Staphylococcus aureus* and *Bacillus typhi* has been tested.

Experiment 1.—Into the peritoneal cavity of a guinea-pig were injected three cultures on agar-agar of *Staphylococcus pyogenes aureus* suspended in 3 c.c. of 0.85 per cent. sodium chloride. Death occurred in twenty-four hours; the peritoneal cavity contained 10 c.c. of turbid fluid from which clear serum was obtained by centrifugalization. The blood serum of a normal guinea-pig and 0.85 per cent. solution of sodium chloride were used for comparison. Since washed leucocytes of the guinea-pig are obtained with difficulty, corpuscles of the dog were employed. Bacteria used were suspended in 0.85 per cent. solution of sodium chloride. Each mixture contained equal volumes of its various ingredients. The following figures represent the average number of bacteria in a leucocyte after incubation at 38° C. during fifteen minutes.

With *Staphylococcus pyogenes aureus*:

Corpuscles + blood serum + staphylococci	16.0
Corpuscles + exuded serum + staphylococci	2.0
Corpuscles + salt solution + staphylococci	3.7

With *Bacillus typhi*:

Corpuscles + blood serum + <i>B. typhi</i>	8.44
Corpuscles + exuded serum + <i>B. typhi</i>	0.16
Corpuscles + salt solution + <i>B. typhi</i>	0.48

To determine if the serum of the exudate was capable of preventing phagocytosis in the presence of blood serum, phagocytosis in a mixture containing two volumes of normal blood serum was compared with phagocytosis in a similar mixture in which one volume of blood serum was replaced by a volume of exuded serum.

Corpuscles + blood serum + blood serum + staphylococci	9.2
Corpuscles + blood serum + exuded serum + staphylococci	11.5
Corpuscles + blood serum + blood serum + <i>B. typhi</i>	6.6
Corpuscles + blood serum + exuded serum + <i>B. typhi</i>	7.0

The experiment shows that opsonin for both *Staphylococcus pyogenes aureus* and for *Bacillus typhi* are absent in the exudate produced by intra-peritoneal injection of the first named micro-organism. The exudate thus produced (containing so-called ag-gressin) fails to inhibit phagocytosis when opsonin is supplied by addition of normal blood serum.

A second experiment with an exudate obtained by injecting the bacillus of swine plague into the pleural cavity of the rabbit gave a similar result. It is noteworthy that this organism, virulent for the

rabbit, failed to undergo phagocytosis in the presence of the blood serum of a normal rabbit, yet almost completely deprived the exudate of power to promote phagocytosis of *Staphylococcus pyogenes aureus* or of *Bacillus dysenteriae*.

Experiment 2.—Into the right pleural cavity of a rabbit was injected one culture on agar-agar of *Bacillus sui pestis*; death occurred in about twenty hours. The exudate present in the pleural cavity was centrifugalized and tested with *B. sui pestis*, *Staphylococcus pyogenes aureus* and *B. dysenteriae*. Blood serum of a normal rabbit and washed corpuscles of the dog were used. Each mixture contained four equal volumes as follows:

With *Bacillus sui pestis*:

Corpuscles + blood serum + salt solution + <i>B. sui pestis</i>	0
Corpuscles + exuded serum + salt solution + <i>B. sui pestis</i>	0
Corpuscles + blood serum + exuded serum + <i>B. sui pestis</i>	0.04

With *Staphylococcus pyogenes aureus*:

Corpuscles + blood serum + salt solution + staphylococci.....	10.4
Corpuscles + exuded serum + salt solution + staphylococci.....	2.2
Corpuscles + blood serum + exuded serum + staphylococci.....	22.7

With *Bacillus dysenteriae*:

Corpuscles + blood serum + salt solution + <i>B. dysenteriae</i>	2.4
Corpuscles + exuded serum + salt solution + <i>B. dysenteriae</i>	0.0
Corpuscles + blood serum + exuded serum + <i>B. dysenteriae</i>	0.3

With one exception (*B. dysenteriae*, Experiment 2) more active phagocytosis has occurred in the presence of a mixture of both blood serum and exudate than in blood serum alone, and this increased activity has been observed even when a mixture containing one volume of blood serum and one volume of exuded serum has been compared with a mixture containing two volumes of blood serum. The following experiment confirms this observation.

Experiment 3.—In the peritoneal cavity of a guinea-pig, twenty-four hours after injection of four agar-agar cultures of *B. typhi*, were found 10 c.c. of turbid fluid. The following tests were made after centrifugalization, the blood serum of a normal guinea-pig and the washed leucocytes of a dog being used.

With *Bacillus typhi*:

Corpuscles + blood serum + salt solution + <i>B. typhi</i>	14.0
Corpuscles + blood serum + exuded serum + <i>B. typhi</i>	21.2

With *Staphylococcus pyogenes aureus*:

Corpuscles + blood serum + salt solution + staphylococci	16.0
Corpuscles + blood serum + exuded serum + staphylococci	26.1

The exuded serum was not tested with bacteria alone until it had stood for some time and the results are not therefore comparable with the foregoing.

Corpuscles + exuded serum + <i>B. typhi</i>	0.2
Corpuscles + exuded serum + staphylococci	3.74

Evidence that absence of opsonic activity may bear no relation to bacterial injection is furnished by examination of the sterile pus obtained by repeatedly injecting small quantities of turpentine into the pleural cavity of the dog.

Experiment 4.—Serum of pus was obtained by centrifugalization of pus removed from the pleural cavity of the dog after repeated injection of turpentine. Absence of bacteria in the exudate was demonstrated by the negative result of attempted cultivation and by examination of stained preparations. The opsonic content of the fluid part of the pus was tested by the usual method, washed corpuscles of the dog and a suspension of *Staphylococcus pyogenes aureus* being used. For comparison, activity of phagocytosis in the presence of blood serum of the dog and in physiological (0.85 per cent.) salt solution was determined.

Corpuscles + blood serum + staphylococci	5.5
Corpuscles + exuded serum + staphylococci	0.5
Corpuscles + salt solution + staphylococci	0.4

The following experiment confirms that just described and shows, moreover, that diminution of phagocytosis is due to loss of opsonic action and not to the inhibiting action of turpentine.

Experiment 5.—An abscess was produced by injecting one cubic centimeter of turpentine into the subcutaneous tissue of the dog. Serum was obtained by centrifugalization from the thick sterile pus present at the end of four days. The opsonic activity of this serum was compared with that of the blood.

Corpuscles + blood serum + staphylococci	1.9
Corpuscles + exuded serum + staphylococci	0.5
Corpuscles + salt solution + staphylococci	0.1
Corpuscles + blood serum + blood serum + staphylococci	2.7
Corpuscles + blood serum + exuded serum + staphylococci	2.8
Corpuscles + exuded serum + exuded serum + staphylococci	1.2

Phagocytosis with serum of the exudate is much less than with blood serum, but when serum of the exudate is mixed with blood serum, phagocytosis is undiminished.

With the serum of the sero-fibrinous pleurisy caused by turpentine injected into the pleural cavity phagocytosis is approximately equal to that which occurs in the presence of blood serum. The following experiment is cited to show that turpentine does not necessarily destroy opsonic activity.

Experiment 6.—Serum from a sero-fibrinous exudate withdrawn from the pleural cavity of the dog three days after injection of 2 c.c. of turpentine was freed from cells by centrifugalization.

Corpuscles + blood serum + staphylococci	10.7
Corpuscles + exuded serum + staphylococci	11.1

The experiments show that the serum of a purulent exudate obtained after the cells have been deposited by centrifugalization may contain no opsonin for staphylococci, even though it has been produced by a sterile irritant and contains no microorganisms. This fact is in agreement with the observation made upon exudates caused by bacteria injected into the guinea-pig and rabbit, for here injection of one microorganism caused loss of opsonin for the other microorganisms which were tested. Nevertheless the experiments do not demonstrate absence of specificity on the part of opsonins, and in Experiment 3 a certain degree of specificity is perhaps suggested, injection of typhoid bacillus causing almost complete disappearance of opsonin for this microorganism, but only partial disappearance of opsonin for staphylococcus.

Absence of opsonin in the exudate produced by an inflammatory irritant of great activity has no necessary relation to the nature of the irritant, for in a purulent exudate caused by a sterile irritant there may be disappearance of opsonin for staphylococci. It is not improbable that the complement-like opsonin of normal sera, which, as Muir and Martin⁴ have shown, is absorbed by a great many substances exhibiting combining affinities for complement, has been absorbed in the experiments just described not only by microorganisms but by cells contained in immense number in the purulent exudate, for it is well known that various tissue extracts can absorb complement.

The observations which have been described probably explain in part the presence of the innumerable extracellular bacteria which may be found in the serum of many purulent exudates. With a microorganism such as the meningococcus, streptococcus and staphylococcus, which readily undergoes phagocytosis in the body or *in vitro*, it is possible that exhaustion of opsonic content of the fluids, rather than impaired phagocytic power of the cells, may explain the occurrence of uningested microorganisms in considerable number in the exudate produced by these bacteria.

⁴ *British Med. Jour.*, 1906, ii, 1783; *Proc. Roy Soc.*, 1907, B. lxxix, 187.

EXPERIMENTAL LIVER NECROSIS; I. THE HEXON
BASES

BY HOLMES C. JACKSON, PH.D., AND RICHARD M. PEARCE, M.D.

EXPERIMENTAL LIVER NECROSIS; I. THE HEXON BASES.¹

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This communication is the first of a series of five, which presents the results of a somewhat comprehensive investigation of the chemical changes associated with experimental liver necrosis and which includes, in addition to that here presented, studies of the nitrogenous metabolism, nuclein metabolism, the activity of the intracellular hepatic enzymes and the changes in the fats and lipoids of the liver.

The advantage of undertaking such a comprehensive study was suggested by the investigations² which one of us had previously made of the necrosis caused in the liver of the dog by the intravenous or intraperitoneal injection of hæmagglutinative and hæmolytic immune sera. The lesions so produced are frequently focal and resemble in a general way those of eclampsia while the diffuse lesions, with the associated repair, are more or less similar to certain stages of acute yellow atrophy. It seemed plausible therefore that a study of the chemistry of such lesions, readily produced experimentally, might throw light not only on certain functions of the liver but might offer new knowledge of value in explaining

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²Pearce, R. M., The Experimental Production of Liver Necroses by the Intravenous Injection of Hæmagglutinins, *Jour. of Med. Research*, 1904, xii, 329; 1906, xiv, 541. Experimental Cirrhosis of the Liver, *Jour. of Exper. Med.*, 1906, viii, 64.

some of the problems of eclampsia, acute yellow atrophy and similar lesions in man.

The study of the hexon bases was undertaken in the hope of determining the character of the processes underlying the production of *intra vitam* autolysis. We have therefore attempted to determine the relative enzymotic power of the normal and the necrotic liver in regard to the synthesis or decomposition of the hexon bases. The importance of these bodies, as products of autolysis, has been emphasized by Wakeman³ in his study of the livers of dogs poisoned with phosphorus or repeatedly anæsthetized with chloroform. In addition to the study of fresh tissues, we have investigated also the changes occurring in hexon nitrogen during antiseptic autolysis of liver substance.

The experimental lesions under consideration may be described, without going into detail, as hyaline necroses with little or no leucocytic reaction, the position and extent of which vary according to the amount of serum administered and the resistance of the animal. Small doses cause focal lesions more or less isolated and irregularly distributed; large doses produce a diffuse necrosis which spares only the tissue about the larger portal spaces. The lesions are found chiefly near the surface of the liver, but may occur in the deeper portions. In animals dying within a few hours after injection and before the appearance of necrosis an intense congestion of the entire portal system exists with, in the liver, innumerable thrombi composed of fused red cells.

In addition to such material from the dog, the livers of horses presenting degenerative and necrotic lesions, occurring in the course of immunization with various bacterial products, have also been utilized. These lesions, which have been described fully elsewhere by Pease and Pearce⁴ and more recently by Lewis,⁵ consist of widespread necrosis associated not infrequently with an extensive deposition of amyloid and usually accompanied by hæmorrhages.

³Wakeman, A. J., On the Hexon Bases of Liver Tissue under Normal and Certain Pathological Conditions, *Jour. of Exper. Med.*, 1905, vii, 292.

⁴Pease, H. D., and Pearce, R. M., Liver Necrosis and Venous Thrombosis in Horses Actively Immunized with Diphtheria and Tetanus Toxins and with Streptococci and their Products, *Journ. of Infect. Diseases*, 1906, iii, 619.

⁵Lewis, P. A., Hæmorrhagic Hepatitis in Antitoxin Horses, *Jour. of Med. Research*, 1906, xv, 449.

A series of fifteen livers, eleven from the dog and four from the horse, has been examined. Of the former, five were normal, four showed focal necroses and two diffuse necrosis; of the latter two were normal and two were examples of necrosis associated with extensive amyloid transformation.

Methods.—As the necrotic lesion in the dog's liver reaches its greatest extent in twenty-four to forty-eight hours after injection, all livers used in the study of the hexon bases were taken from animals killed at the end of the latter period. In the case of the horses the livers were removed within one hour after death.

After cutting away the larger vessels and the coarser tissue about the hilum the organ was subdivided and passed through the hashing machine. Small portions were weighed and set aside for drying and upon these total nitrogen estimations were made later. At the same time two portions of 100 grams each of moist liver were placed in flasks. To one, 600 cubic centimeters of water and 300 grams of sulphuric acid were added and hydrolysis carried on for fourteen hours in a paraffin bath. The other sample was suspended in 600 cubic centimeters of water and with it were thoroughly mixed about fifty cubic centimeters of toluol. This flask plugged with cotton was allowed to stand at room temperature for periods of one or two months. At the end of this time it was examined bacteriologically, and if found free of bacteria, 300 grams of sulphuric acid were added and the material hydrolyzed in the same manner as the control. Subsequent treatment was carried out according to the method outlined by Wakeman except that the process was continued only to the point of making nitrogen determinations of fractions "A" (arginin and histidin) and "B" (lysin) after removal of the barium and silver by means of sulphuric acid and hydrogen sulphide. For our purpose no advantage was to be gained by separating arginin and histidin.

Dry Solid Content.—An examination of the figures in the table indicates that the dry solid content of the dog livers with scattered focal necroses did not vary from the normal average of 24 per cent. In the case of the diffuse lesions, where the autolysis of the protoplasm was obviously more marked, the dry solids were reduced to an average of 20.8 per cent. The figures referring to normal livers

TABLE--Heron Bases.

Nature of Experiment.			Determination on Fresh Tissue.						On Autolysed Tissue.									
Dog.	Condition of Liver.	Per Cent.		* Per Cent. Nitrogen as				Per Cent. of N, ppt. by Phosphotung. Acid.	* Per Cent. Nitrogen as									
		Dry Substance.	Nitrogen in Dry Substance.	N, ppt. by Phosphotung. Acid.	Arginin Histidin.	Lysin.	Purin Bases.		Arginin and Histidin.	Lysin.	Purin Bases.							
3	Normal	24.8	12.1	13.2	4.3	32.5	3.6	27.1	5.3	40.4	Not bacteria-free	8.4	42.4	9.8	1.6	8.1		
4	Normal	18.4	10.9	15.0	6.0	40.0	4.9	32.7	4.1	27.3		19.8	8.0	43.7	7.3	3.0	16.4	
13	Normal	26.8	11.8	Used only for autolysis			Used only for autolysis			Used only for autolysis			7.5	42.6	7.6	2.5	14.2	
14	Normal	24.5	9.2	Used only for autolysis			Used only for autolysis			Used only for autolysis			8.0	39.2	7.9	4.5	22.1	
17	Normal	25.0	11.2	19.6	4.3	36.2	7.4	37.7	5.1	26.1		20.4	8.0	42.0	8.2	2.9	15.2	
Av.		23.9	11.3	15.9	6.0	36.2	5.3	32.5	4.8	31.3	19.0	8.0	42.0	2.7	30.0	2.0	24.5	
5	Few focal necroses	24.4	21.4	12.2	5.6	45.8	4.2	34.4	2.4	19.8	9.0	4.1	45.5	3.5	34.3	1.9	17.7	
7	Few focal necroses	16.6	19.1	14.5	7.3	50.2	5.1	35.1	2.1	14.7	10.2	4.9	48.0	2.6	38.8	1.8	26.9	
9	Numerous focal necroses	26.9	22.8	10.2	4.8	47.2	3.2	31.5	2.1	21.3	6.7	2.3	34.3	1.9	27.9	2.1	32.3	
10	Numerous focal necroses	25.6	23.7	8.5	3.6	42.3	2.5	29.4	2.4	28.3	6.8	2.7	39.8	2.7	32.8	2.0	25.3	
Av.		23.4	21.8	11.4	5.3	46.4	3.8	32.6	2.2	21.0	8.2	3.5	41.9	10.2	42.7	2.2	9.2	
29	Extensive diffuse necrosis	21.9	12.7	30.0	15.6	52.0	11.6	38.6	2.8	9.4	23.9	11.5	48.1	9.5	45.9	1.1	5.3	
60	Extensive diffuse necrosis	19.8	13.2	25.6	12.7	49.6	11.3	44.1	1.6	5.3	20.7	10.1	48.8	9.9	44.3	1.7	7.3	
Av.		20.8	12.95	27.8	14.2	50.8	11.5	41.4	2.2	7.4	22.3	10.8	48.5	9.9	44.3	1.7	7.3	

TABLE—Heron Bases (Continued).

Nature of Experiment.		Determination on Fresh Tissue.					
Horse.	Condition of Liver.	Per Cent.			* Per Cent. Nitrogen as		
		Dry Substance.	Nitrogen in Dry Substance.	N ₂ ppt. by Phosphotungstic Acid.	Arginin and Histidin.	Lysin.	Purin Bases.
60	Normal	23.3	12.0	8.1	3.0	4.6	0.5
65	Normal	21.8	11.2	8.5	3.9	56.7	6.3
Av.		22.6	11.6	8.3	45.9	36.5	17.6
42	Necrosis and amyloid	20.0	21.7	13.5	3.5	46.6	0.75
69	Necrosis and amyloid	21.4	13.7	13.3	41.5	8.2	12.0
Av.		20.7	17.7	13.4	21.4	60.7	2.4
					9.0	10.2	17.9
					2.1	76.7	1.9
					15.2	9.2	14.3
						68.7	2.2
							16.1

* Figures in upper left hand corner are calculated on the total nitrogen; those in the lower right hand corner on the total hexon nitrogen.

agree somewhat closely with those of Wakeman, who, however, noticed a slight decrease in the percentage of dry substance in the livers of dogs poisoned by phosphorus. This is somewhat surprising since he describes these livers as markedly fatty. If the protoplasm of which seventy-five per cent. is water is replaced by fat, which contains none, the dry solids should increase instead of decrease. If Wakeman's figures are correct they show a marked increase in the water content of the organs during phosphorus poisoning. That such an increase may occur did not appear in our investigation⁶ of fatty changes in the liver.

Total Nitrogen Content.—Several interesting facts developed from the analysis of the nitrogen of the dry solids. The average of the five determinations for normal tissue was 11.3 per cent. in agreement with Wakeman; while that of the livers with lesions of a scattered focal character was 21.8 per cent.

This, of course, indicates a deposition or heaping-up of nitrogenous material in the hepatic cell. That such a process may take place even under physiological conditions is evident from the results of experiments carried out by Seitz.⁷ This investigator found that by feeding hens and geese excessive amounts of meat a true deposition of nitrogenous substances occurred in the cells of the liver. This increase amounted in some instances to 300 per cent.

In our experiments the quantity of nitrogen in the hepatic cell was almost doubled in the organs with scattered necrotic lesions. This condition allows of an explanation similar to that offered for the infiltration of fat in tissues during phosphorus or phloridzin poisoning. In such lesions the cells have lost in part their power to oxidize properly the sugar or other materials placed at their disposal by the circulating blood and hence the starving cell, in its endeavor to spare its own protoplasm from destruction, stores up fat for purposes of oxidation.

That the cells at the margin of the necroses under consideration do accumulate fat we have observed in our study of the histological changes occurring in these livers.⁸ It seems distinctly possible that

* See fifth paper of this series, "The Fats and Lipoids" in this number of the *Journal*.

⁷ Seitz, W., Die Leber als Vorrathskammer für Eiweissstoffe, *Arch. f. ges. Physiol.*, 1906, cxi, 309.

during the initial stage of congestion and thrombosis and in the early stages of necrosis, the imperfectly nourished and slightly injured cells may heap up nitrogenous material also. When, however, the lesion is more extensive, the storing up of nitrogen is not so evident as shown by the fact that the nitrogen of the dry solids remains more nearly normal. This is to be explained by assuming that the nitrogen stored up in the persisting liver cells is sufficient to more than balance the loss by autolysis in the necrotic areas.

Wakeman's figures for the nitrogen content of livers after phosphorus poisoning show a diminution equivalent to 35.6 per cent. and a corresponding decrease in the hexon base nitrogen. This indicates, according to his view, that that part of the proteid molecule involving the hexon bases has not undergone a relatively greater decomposition than the other nitrogenous substances. It would seem to us that the low nitrogen content of the phosphorus livers is wholly, or in greatest part, due to the large amount of fat present.

The Hexon Bases.—Wakeman's results indicate that in the dog the average nitrogen content of the bases in the normal liver tissue is 17.04 per cent. of the total nitrogen, while in the liver of dogs poisoned with phosphorus it is only 10.72, a falling off of 37.1 per cent. The livers of dogs receiving chloroform showed 13.6 per cent., a decrease of 20 per cent. In these figures Wakeman sees evidence of increased autolysis in hepatic cells affected by phosphorus or chloroform. Although he mentions definite necrosis in but one of his livers, the cell destruction in phosphorus poisoning, gradual as it is, is such that our results ought to fall, as they do, somewhat into line with his.

Thus as an average of three normal livers we find the figures concerning the total content of hexon bases, based on nitrogen content attributable to them, to be 15.9 per cent. of the total nitrogen. In the case of the scattered focal necrosis, the percentage is slightly decreased to 11.4 as an average of four determinations, but the absolute amount is increased. That is gram for gram of dry substance there occurs an increase in the absolute amount of hexon bases which however appears as a decrease in percentage on account of the high nitrogen content of the dry substance.

This absolute increase in hexon content of dry substance is greater and more clearly accentuated in the livers of those dogs in which the necrosis is more diffuse. Here the nitrogen of the dry substance is almost the same as that of the normal, 12.95 per cent. as average of two determinations; but the hexon base nitrogen content rises to 27.8 per cent. of the nitrogen of the dry substance.

This observation is extremely interesting in that it points most strongly to the preponderance of the autolytic process over the synthetic in the more widespread forms of necrosis with early repair. The figures show a definite increase in the hexon base content of the necrotic cell, although the accumulation of nitrogen in this lesion, 12.95 per cent., could not occur to such a marked extent as it did in the focal lesion, 21.8 per cent., because of the lessened number of persisting living cells capable of storing up nitrogen. A rearrangement of nitrogen, the result of autolysis in the larger areas of necrosis, therefore took place as shown by the hexon nitrogen content of 27.8 per cent., as compared with that of 11.44 per cent. in the focal lesion and 15.9 per cent. in the normal. This great increase of hexon bases may be due in part also to disturbances of the circulation accompanying the necrosis which prevent the diffusion and removal of the bases from the liver. In this connection attention may be called to Jacoby's⁸ observation that leucin and tyrosin are not found in the liver of phosphorus poisoning when no disturbance of the hepatic circulation exists.

Relation of Precipitate "A" (Arginin and Histidin) and Precipitate "B" (Lysin) to the Total Hexons.—Wakeman, from a consideration of his results on these fractions, concludes that in the autolysis which occurs in the cell in phosphorus poisoning the arginin suffers a greater destruction than do the other bases, probably through the action of arginase, which splits arginin into ornithin and urea.⁹ His tables show that of the 17.0 per cent. of the nitrogen of the total bases in the normal tissues, 11.8 per cent. is to be attributed to arginin and histidin and 5.2 to lysin. In phosphorus poisoning, on the other hand, the nitrogen of the bases amounts to

⁸ Jacoby, M., Ueber die Beziehungen der Leber und Blutveränderungen bei Phosphorvergiftung zur Autolyse, *Zeit. f. physiol. Chem.*, 1900, xxx, 174.

⁹ Kossel, A., and Dakin, H. D., Ueber die Arginase, *Zeit. f. physiol. Chem.*, 1904, xli, 321.

10.7 per cent., of which only 6.8 per cent. belongs to the arginin and histidin and 3.8 per cent. to the lysin. This indicates a decrease during autolysis of 42.3 per cent. for the arginin and histidin, but only 26.8 per cent. for the lysin.

If, however, one considers these figures from the standpoint of the relationship which the precipitates "A" and "B" bear to the total hexon bases of the normal and phosphorus dogs, an entirely different view is obtained. In the normal tissue precipitate "A" (arginin and histidin) forms 69.6 per cent. and precipitate "B" (lysin) 30.4 per cent. of the total bases; whereas in the phosphorus livers the former is 63.9 per cent. and the latter 36.1 per cent. Hence the decrease in the fraction "A" is only 5.7 per cent. and this is offset by the corresponding increase in fraction "B." This diminution in the arginin and histidin content of the hexon base fraction of the livers of phosphorus-poisoned animals compared with the normal is so slight that it hardly seems warrantable to attribute it to the action of arginase.

Our figures for the total hexon bases (15.9 per cent.) in the normal agree well with those found by Wakeman, while those for the focal necroses have suffered a percentage decrease which is somewhat comparable to that noticed by him in phosphorus poisoning. Wakeman's percentage decrease, however, was also an absolute one while ours in reality was an absolute increase, as has been explained above. The proportion which precipitates "A" and "B" bear to the total is markedly different, however, from that which he notes. Our average in the normal tissues for the arginin and histidin fraction is 36.2 per cent. as against 69.4; the lysin fraction 32.5 per cent. more nearly agrees with his 30.3 per cent. If we exclude the purin bases our results become more comparable and agree better in percentage.

We have also found a much greater percentage of the total nitrogen due to purin bases. Wakeman's figures show an average of 0.0273 per cent. for normal and pathological, while ours showed 4.8 per cent. for the normal tissue against 2.2 per cent. for the focal necrotic lesions and the same for the diffuse necrosis.

As to variations which the individual bases undergo in their relation to the total bases, our results point to an increase from the

normal of 36.2 per cent. to 46.4 during the focal necrosis and to 50.8 per cent. during diffuse necrosis for arginin and histidin. The lysin fraction shows no change in the focal necroses as compared with the normal, but in the diffuse necrosis it increased 24.3 over the normal in agreement with Wakeman.

In the autolysis *in vitro*, which is discussed in the next paragraph, the normal fraction "A" represented 42.0 per cent. of the whole bases and fraction "B" 42.8 per cent.—differences from the normal which are well within the limit of error. Hence the absolute increase from 6.0 and 5.3 in the unautolyzed to 8.0 and 8.2 per cent. of the total nitrogen in the tissue after autolysis was in exact relation to the increase in total hexon nitrogen. The same is true for the autolyzed tissue with necrosis of all types.

This would emphasize more markedly the point made above that small evidence can be adduced to show that an enzyme, arginase, is acting on the arginin, decreasing its amount during autolysis. Such action is not shown by our figures and moreover in our investigation of intracellular hepatic enzymes¹⁰ we could not obtain an active arginase from the necrotic dog's liver, though it was found in the normal.

Hexons Resulting from Autolysis in Vitro.—It seemed worth while in view of the investigation, carried on synchronously, of intracellular hepatic enzymes¹⁰ to determine the relation of the hexon bases to autolysis of the liver *in vitro*. For such observations the figures given above for autolysis during life serve as controls. Autolysis was allowed to proceed for varying lengths of time in the endeavor to determine whether the different organs showed varied degrees of autolysis. The periods selected, one and two months, were inadequate to bring out this point, since the autolysis was completed or the reaction reached its equilibrium before one month. This was unfortunate as we thereby disregarded the important element, that of time, in this connection. The time element, however, is fully considered elsewhere¹⁰ from another point of view.

A glance at the figures in the table shows that after autolysis of the normal organs the percentage of total nitrogen as hexon

¹⁰ See second paper of this series, "Enzymes," in this number of the Journal.

nitrogen was 19.0 per cent. as an average of four determinations. This increase of 18.8 per cent. over the normal hexon content is not marked, and if one examines the figures referring to the two dogs (4 and 17) upon which alone we have absolute controls, it will be seen that this increase is variable.

We are not inclined to attempt to explain this result in detail in this place, since the data which we will present in our study of the enzymes bear more decisively upon this matter. Suffice it to say that these figures, taken in connection with those of the diffusely necrotic organs where an increase also was evident, indicate that in the autolysis a transformation or rearrangement occurs by which nitrogenous atomic complexes, not normally yielding hexon bases, become altered into hexon bases or their combinations.

In all degrees of necrosis, the autolyzed material contained a smaller amount of hexon bases than the unautolyzed. In the liver with scattered focal necroses the decrease of the hexon nitrogen in per cent. of the total nitrogen amounted to 28.0 per cent.; in the diffuse necrosis 19.8 per cent. This would seem to imply that in the living tissue the hexon splitting enzyme is to some degree inhibited, probably through the action of the blood serum.

Hexon Bases in the Liver of the Horse.—The results obtained with the normal livers of horses agree in regard to the dry solids (22.6 per cent.) and the nitrogen of the dry tissue (11.6 per cent.) with those obtained for the dog. The nitrogen precipitable with phosphotungstic acid, however, is surprisingly low, amounting to only 8.3 per cent. It would seem inadvisable to attempt an explanation of this difference, since these animals were not absolutely normal, in that they had been utilized for the purpose of preparing antitoxin and had died during such treatment, though no lesions were found in the liver. The injection of bacterial products may set up processes in the cell which tend to reduce its hexon content without changes evident histologically. The percentage which fractions "A" and "B" of such livers bears to the total hexon content is not far removed from that found for each in the case of the normal dog liver.

The two lesions which served as examples of necrosis presented the complicating feature of amyloid. This fact renders the series

not exactly comparable to the previous one. The increase in nitrogen of the dry substance over that of the normal is present here also, and the total hexon base nitrogen has increased to 13.4 per cent. This latter result may be caused by the heaping up of the bases as products of autolysis in the large necrotic areas. More probably, however, it is to be attributed to the amyloid degeneration, in which the normal cellular proteids with relatively small percentage (10–30 per cent.) of diamino-nitrogen are replaced by amyloid, the proteid constituent of which, in the liver and spleen as shown by Neuberg,¹¹ contains twice as much (50–60 per cent.) diamino-acid nitrogen. This rearrangement in the hexon content of the proteids of the organ has also resulted in a marked change in the relation which the arginin and histidin as well as the lysin bears to the total amount of hexon base precipitate. “A” forms only 15.2 per cent. and precipitate “B” 68.7 per cent. of the whole diamino-acid nitrogen.

SUMMARY.

1. The liver of the dog in which necrosis has been produced by injection of hæmatotoxic immune sera is characterized in the less marked forms by a storing up of nitrogen in the persisting living cells, while in the diffuse forms the total nitrogen content is but slightly above the normal. This last is to be explained by the great diminution in persisting liver substance which limits the power of nitrogen accumulation.

2. In all forms of necrosis there occurs an absolute increase of nitrogen precipitable by phosphotungstic acid (hexon bases) but the percentage increase, in relation to total nitrogen, diminishes in those forms (focal) in which the products of autolysis may be readily carried off by the blood stream and greatly increases in the diffuse form with large areas in which the circulation is seriously impaired.

3. Although the absolute amount of nitrogen representing arginin and histidin varies, a relative increase is evident when this fraction is compared with the total diamino-nitrogen. This increase corresponds to the degree of necrosis and attendant circu-

¹¹ Neuberg, Ueber Amyloid, *Verhand. d. Deut. path. Gesellsch.*, 1904, vii, 19.

latory disturbance and indicates that in necrosis as opposed to degeneration (Wakeman) arginin is not split up by arginase. The lysin also bears a definite relation to the total hexon nitrogen.

4. The diamino-nitrogen of the normal liver after autolysis *in vitro* shows a slight variable increase over that of the unautolyzed, while the necrotic livers showed a decided decrease.

5. The diamino-acid nitrogen of normal horse liver is only about one half of that of the dog; the relative proportion of the bases is about the same. In necrotic livers with amyloid the diamino-nitrogen is markedly increased which is in accord with Neuberg's observations on the high hexon base content of amyloid.

Conclusions.—Upon the whole then the chemical processes occurring in the hepatic cell undergoing rapid or immediate necrosis and those accompanying a slow "degeneration," as for example in phosphorus poisoning, must be different and distinct as would be expected from the histological findings. In necrosis we find the cell in a complete state of disorganization and decomposition and hence autolysis begins immediately, but in the changes occurring in the cell in the so-called degenerations, as phosphorus poisoning, the nucleus remains intact, thereby insuring to a certain extent the life or at least partial function of the cell. That under the latter circumstance a disturbed condition does exist is evidenced by the heaping up of fat in the cell, and although the results of the various investigations upon the altered processes in the liver of animals poisoned with phosphorus tend to show that this change is an autolysis, in which certain amino-acids appear as the result of the splitting of the proteid molecule, it is not of the same type as that appearing in the necrotic cell. This is shown by a comparison of Wakeman's findings, which indicate definitely a diminution of hexons in the liver, with ours which show a great increase.

Examinations of the human liver, by a direct method without hydrolysis, but few in number, it is true, tend to the same conclusion. Taylor,¹² for example, found arginin in a liver with wide-

¹² Taylor, A. E., Ueber das Vorkommen von Spaltungsprodukten der Eiweisskörper in der degenerirten Leber, *Zeit. f. physiol. Chem.*, 1902, xxxiv, 580. On the Occurrence of Amino-acids in Degenerated Tissue, *Univ. of California Publications*, 1904, i (Path.), 43.

spread necrosis, the result apparently of chloroform poisoning; from seven other livers, representing various lesions (dysenteric abscess, fatty degeneration, pyæmia and acute yellow atrophy), he was unable to isolate this substance. Soetbeer¹³ also was unable to find hexon bases in a peculiar type of cirrhosis with acute degeneration.

This difference in the hexon content of the liver of "degeneration" and that of necrosis is so striking that it would appear to be due to a difference in the nature or rapidity of the cell destruction, though it may to some extent be explained by the disturbances of circulation which occur in the necrosis and which, presumably, are absent in degeneration.

Our observations show also that care must be exercised in drawing conclusions from results obtained by autolysis *in vitro*. It seems distinctly doubtful whether the autolysis of the cell which occurs under such circumstances has any relation to autolytic changes during life.

¹³ Soetbeer, F., Ueber einen Fall von akuten Degeneration des Leberparenchyms, *Arch. f. exper. Path. u. Pharm.*, 1903, 1, 294.

EXPERIMENTAL LIVER NECROSIS; II. ENZYMES

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EXPERIMENTAL LIVER NECROSIS; II. ENZYMES.¹

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The experiments about to be described represent an attempt to determine the relation of the intracellular hepatic enzymes to chemical changes occurring in liver necrosis. Our results are based on a comparison of the variations in the enzymotic equilibrium of the normal hepatic cells with those occurring in necrosis of varying grades of severity. At present the chief and most promising method of detecting such variations consists in determining by means of post-mortem autolysis the condition under which the cell is existing at the time of the death of the animal, and the rapidity, nature and extent of the changes which occur after the commencement of the autolysis. We are well aware that the interpretation of the results of post-mortem autolysis in relation to cellular activity during life is open to objection and may not have the importance usually ascribed to it.

Our investigation of the enzymotic activity of the liver tissue under normal circumstances and in varying degrees of necrosis may naturally be subdivided as follows:

1. A determination in a quantitative way of the degree of autolysis which the tissue undergoes after death.
2. A study of the individual enzymes with reference to the part which they play in the general course of autolysis.

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3. A determination of the products formed as the result of such autolysis. These include the diamino-acids, which have been considered in the preceding paper,² where they more properly belong, and the monamino-acids to which, as represented by leucin and tyrosin, we have given considerable attention. It was our intention to determine, by perfusion of livers in various stages of necrosis, the changes in the composition of the blood which might occur, but owing to the great amount of labor entailed in the present studies this has been unavoidably postponed.

Comparative Estimation of Products of Autolysis.—In the study of the changes which the nitrogenous material undergoes during autolysis *in vitro* an attempt was made to carry out a partition analysis of the non-coagulable nitrogen. This method has already been employed with good results by v. Drjewezki³ to determine the effect of alkalies of varying strengths upon autolysis. His results, as well as those of Wiener,⁴ point to the sensitiveness of the autolytic enzymes to changes in reaction, especially those due to alkalies, and these investigators conclude that the alkalies of the serum are responsible for the well-known inhibitory effect of the serum upon autolysis. Baer⁵ and his associate, Loeb,⁶ admit the inhibitory effect of the serum but are inclined to attribute it to the action of the serum globulin.

These facts, as well as those brought out by Lang⁷ concerning the inhibitory effect of large quantities of toluol upon autolytic processes, although other factors may have influenced the results of the latter, all tend to emphasize the fact that in performing experiments of this character too much attention cannot be given

² See first paper of this series, "Hexon Bases" in this number of the *Journal*.

³ v. Drjewezki, A., Ueber den Einfluss der alkalischen Reaktion auf die autolytischen Vorgänge in der Leber, *Biochem. Zeit.*, 1906, i, 229.

⁴ Wiener, H., Ueber den Einfluss der Reaktion auf autolytische Vorgänge, *Zent. f. Physiol.*, 1905, xix, 349.

⁵ Baer, J., Ueber die Wirkung des Serums auf die intracellularen Ferments, *Arch. f. exper. Path. u. Pharm.*, 1906, lvi, 68.

⁶ Baer, J. and Loeb, A., Ueber die Bedingungen der autolytischen Eiweiss-spaltung in der Leber, *Arch. f. exper. Path. u. Pharm.*, 1905, liii, 1.

⁷ Lang, S., Ueber desamidierung im Tierkörper, *Beit. z. chem. Physiol. u. Path.*, 1904, v, 321.

to the attainment of absolutely comparable conditions in all the various experiments. With these points in mind we have endeavored to control our work in every way, as is shown in the following detail of the experiments.

Quantities of the fresh tissue of known weight were ground to such a state of subdivision that when mixed with water or neutral Ringer's solution the mixture could be readily drawn up in a pipette. This mixture, usually consisting of two hundred grams of liver, was made up to 1,200 cubic centimeters and placed in a sterile flask and the mixture covered with a layer of toluol. This latter substance was well shaken in, after which were pipetted off, as controls, two samples of two hundred cubic centimeters each. Both were thoroughly sterilized in the autoclave in order to stop autolysis; one was examined immediately, as an initial control, the other was placed with the original mixture in the thermostat (37.5° C.) and examined at the conclusion of the experiment as a final control. The material in the thermostat was shaken from time to time and at intervals of one, three, five and eight days samples of the main mixture were removed for analysis by the same method as the controls. The analysis of the samples, after they were shown to be bacteria free,⁸ took place in the following manner:

The mixture was pipetted into a beaker and sufficient water was added to allow of easy coagulation of the proteid material present. Acetic acid was added to slightly acid reaction after the boiling point was reached. The coagulated proteid was removed by filtration and repeatedly and thoroughly washed with boiling water. The volume of the filtrate and washings was made up to eight hundred cubic centimeters. Of this, twenty-five cubic centimeters served for the determination of the total nitrogen by the Kjeldahl-Gunning method, one hundred for the estimation of ammonia according to the method of Shaffer as applied to the urine, one hundred for the uric acid determination, using the Hopkins-Folin method, and fifty to determine the amount of nitrogen not precipitable by phosphotungstic acid in sulphuric acid solution, the so-called

⁸ For this purpose it was deemed sufficient merely to examine stained films though when the final sample of each mixture was taken cultures were made. In this series representing nine livers no contamination occurred.

monamino-nitrogen. An attempt was also made to determine the nitrogen precipitable (proteoses) by zinc sulphate but our results are so incomplete that little can be gained from their discussion. In all cases duplicate determinations were made and the figures given represent their average. Dogs were employed in all experiments.

In Table I are presented the results of the nine experiments which differed in their conditions for the purposes of control, as follows:

Two normal livers (52 and 54) with their usual blood content, the diluting fluid of one being distilled water and of the other neutral Ringer's solution. This solution was prepared in the ordinary way with the exception that the sodium bicarbonate was not added in order to avoid an alkaline medium.

Two normal livers (53 and 58) washed *in situ*, the one with water and the other with neutral Ringer's solution.

One necrotic liver four hours after injection (57). An attempt was made to wash this liver with water but on account of the extensive thrombosis it was only partly successful. It is therefore referred to as "half-washed."

Two necrotic livers forty-eight hours after injection (48 and 56); one unwashed diluted with water; the other washed and diluted with neutral Ringer's solution.

Two livers (43 and 49) five days after injection, both showing necrotic lesions with early repair; one washed and diluted with Ringer's, the other unwashed but diluted with water.

In each instance in which the livers were washed the procedure was begun under ether while the animal was alive. With the exception noted the livers were completely blanched save for slightly tinged areas about the more diffuse foci of necrosis.

The results in Table I, in terms of nitrogen, are expressed in percentages of the total nitrogen of the dry tissue and of the total non-coagulable nitrogen. A critical consideration of the figures presented allows of the following statements:

Non-coagulable Nitrogen.—The inhibiting effect of the blood serum upon the extent of the autolysis of both normal and necrotic tissue is decisively shown. The percentage of non-coagulable nitrogen in the case of the unwashed normal organs increased from 10.7 and 9.7 to 19.9 and 29.1 per cent. respectively, an increase of 100 and 200 per cent., on the eighth day; while in the washed normal livers the average increase at the eighth day amounted to 450 per cent. The increase in the five day necrotic unwashed liver was 127 per cent.; that of the washed tissue 349 per cent. The forty-

TABLE I.
Autolysis; nitrogen partition.

Normal.				4 Hours.	48 Hours. Necrosis.		5 Days. Necrosis.		Dura- tion.
Not Washed.		Washed.		Washed.*	Not Washed.	Washed.	Not Washed.	Washed.	
52†	54	53	58	57	48†	56	43†	49	

Percentage of total nitrogen in non-coagulable form.

10.7	9.7	13.5	9.5	8.5	12.7	8.3	26.6	18.3	Control
15.5	18.6	35.5	23.6	19.3	20.0	18.5	39.4	41.1	1 day
18.2	27.8	57.6	38.5	24.1	24.9	65.1	51.1	48.5	3 days
19.8	27.8	67.5	49.7	29.6	30.8	75.7	54.3	61.0	5 days
19.9	29.1	70.1	54.2	37.9	32.1	79.4	60.4	82.3	8 days
13.4	11.6	14.0	8.9	7.4	14.5	7.2	22.1	18.1	Final Control

Phosphotungstate-filtrate nitrogen (monamino-acid).

7.2	6.7	69.3	7.4	54.8	4.2	44.5	5.5	64.7	10.7	81.1	3.6	43.0	15.0	56.5	11.3	51.7	Control
12.4	80.0	73.6	30.9	87.0	77.1	72.8	14.7	17.2	17.2	86.0	16.4	88.6	29.7	75.4	30.0	73.1	1 day
14.1	77.5	77.0	49.8	86.6	82.6	82.5	19.9	22.6	22.6	90.8	54.7	84.2	37.2	72.8	39.3	81.0	3 days
16.2	81.8	82.4	56.9	84.3	81.0	85.4	25.3	26.0	26.0	84.4	64.3	84.9	43.8	80.7	53.0	86.9	5 days
16.9	84.9	82.7	57.8	80.1	85.2	85.2	32.3	27.2	27.2	84.7	70.1	88.3	46.9	77.6	71.9	87.3	8 days
8.4	63.0	70.0	7.6	54.1	54.3	90.5	6.8	8.9	8.9	75.4	3.7	51.5	11.9	53.8	11.9	66.0	Final Control

Ammonia nitrogen.

0.74	0.49	0.59		0.79	0.80	0.85	2.08	1.10		Control
6.9	5.1	4.3		9.4	6.3	10.2	8.6	6.0		
1.38	0.90	1.41		1.05	1.46	1.50	4.4	2.20		1 day
8.8	4.8	4.0		5.4	7.3	8.1	10.6	5.3		
1.45	1.06	2.45		1.9	2.16	2.78	4.7	3.30		3 days
7.9	3.8	4.2		7.9	8.6	4.3	9.2	6.9		
1.63	1.60	2.52		1.6	2.19	3.25	5.8	3.99		5 days
8.2	5.1	3.8		5.4	7.1	4.3	9.6	6.5		
2.08	1.22	2.45		2.9	2.34	3.01	6.4	5.11		8 days
10.4	4.2	3.5		7.6	7.3	3.8	10.6	6.2		Final
0.66	0.67			1.02	0.85		2.2	1.43		Control
4.9	5.2				8.6	11.6	10.0	7.9		

* Washing incomplete.

† Distilled water used instead of neutral Ringer's solution.

Figures in upper left-hand corner show percentage of total nitrogen; those in lower right-hand corner, of non-coagulable nitrogen.

eight hour washed tissue (Dog 56) with a very extensive necrosis, showed the greatest increase, equivalent to 856 per cent. of the control. The increase of the four hour experiment (congestion and thrombosis) hardly equaled that of the washed normal.

Wherever water was employed in washing or in diluting, the autolysis was distinctly less than when neutral Ringer was used. (Compare Dogs 52 and 54.)

Concerning the rapidity of the autolysis, it may be noticed that, though the initial increase during the first day in the case of the unwashed tissues is but one half of that of the washed, it represents, as does also the increase of the washed, fifty per cent. of the total autolysis. On the third day, however, the autolysis has reached its maximum in the unwashed tissues, while the washed organs continue to increase until the eighth day, when the autolysis in their case is also apparently complete.

In the forty-eight hour lesions in which, histologically, the autolysis of the necrotic areas would appear to be at its height, we see that the autolytic processes *in vitro* were also very active. The increase at the end of the eighth day in the unwashed liver (Dog 48) was about 150 per cent., but after the removal of the inhibitory action of the blood (Dog 56) the increase rose to almost 900 per cent. The same thing is evident in the fifth day lesions but is not so pronounced.

The rapidity with which the autolysis reaches its maximum is of course dependent upon various factors. The attainment of the maximum signifies that the reaction velocities of the system, made up of substrat, hydrolytic agent and enzyme, have reached an equilibrium, caused, no doubt, by the non-removal of the products of autolysis. Since we must assume that the substrat and enzyme are the same in the normal tissues of both washed and unwashed organs, the varying factor must consist in the hydrolysis which, from the work of Wiener, seems undoubtedly due to the unneutralized acids formed during autolysis as first described by Magnus-Levy.⁹ The acids which are formed in the normal metabolism of the cells are neutralized by the ammonia and excess of bases in the blood; hence

⁹ Magnus-Levy, A., Ueber die Säurebildung bei der Autolyse der Leber, *Beit. z. chem. Physiol. u. Path.*, 1902, ii, 261.

autolysis does not occur in the living cell. As soon as the serum with its neutralizing power is removed, as in the washed organs or where the acids use up the excess of bases as in the center of a large area of necrosis, the conditions necessary for autolysis are present and hydrolysis of the substrat protoplasm takes place.

Phosphotungstate Filtrate Nitrogen (Monamino-acids).—The phosphotungstate precipitate has been disregarded here, for, as it consists of diamino-nitrogen it has been sufficiently covered in the autolysis experiments in connection with the study of the hexon bases.¹⁰

By far the major portion of the nitrogen in the filtrate is in the form of monamino-acids.¹¹ The table indicates the percentage of the fraction in terms of the total nitrogen of the tissue as well as of the total non-coagulable nitrogen. Our figures for the controls indicate that in the normal tissue, washed or unwashed, 4.2 to 7.4 per cent. of the total nitrogen is to be attributed to nitrogen not precipitable with phosphotungstic acid. Of the forty-eight hour lesions, that with the most marked diffuse necrosis (Dog 48) showed 10.7 per cent. of the total nitrogen in that form, while the other, of the focal type (Dog 56), had only 3.6 per cent. or slightly less than the lowest of the normal figures. Also, in the first of this pair, 81.1 per cent. of the non-coagulable nitrogen was in the form of monamino-acid while the other showed only 43.0, again somewhat less than normal.

These two experiments illustrate most decisively the point which Taylor's¹² results seem to indicate. That is, there is an absence of monamino-acids in pathological conditions of the liver accompanied by little or no necrosis, while in necrosis of the diffuse type both the monamino- and diamino-acids are present. We have elsewhere¹³ suggested that the relation of circulatory disturbances to

¹⁰ See first paper of this series, "Hexon Bases" in this number of the *Journal*.

¹¹ v. Drjewezki, A., Ueber den Einfluss der alkalischen Reaktion auf die autolytischen Vorgänge in der Leber, *Biochem. Zeit.*, 1906, i, 229.

¹² Taylor, A. E., Ueber das Vorkommen von Spaltungsprodukten der Eiweisskörper in der degenerirten Leber, *Zeit. f. physiol. Chem.*, 1902, xxxiv, 580. On the Occurrence of Amino-acids in Degenerated Tissue, *Univ. of California Publications*, 1904, i (Path.), 43.

¹³ See first paper of this series, "Hexon Bases" in this number of the *Journal*.

the removal of the products of autolysis is an all-important factor. This is further supported by the two experiments under discussion which indicate that the organ with the focal lesions contained no more monamino-nitrogen than did the normal tissue. In this case the circulation was very slightly, if at all, impaired, and these acids, if they were formed, were removed immediately by the blood stream. In Experiment 48 the large necrotic areas, the centers of which were remote from circulatory fluids, held the acids as they were produced. That these substances are produced in autolysis of this type *in vivo* in large quantities is also indicated by the fact that 81.1 per cent. of the non-coagulable nitrogen of this liver was present in the fresh tissue as nitrogen non-precipitable with phosphotungstic acid. This value approaches that found in all the other cases after autolysis *in vitro* has proceeded for from one to three days.

The control figures of the five day necrosis, as shown by Experiments 43 and 49, also indicate a high percentage of the total nitrogen of the tissue as monamino-nitrogen. As, however, an unusually large amount of the total nitrogen occurs in non-coagulable form the percentage relation of the monamino acids to the latter is about normal. These lesions were very extensive but of the focal type and the cells at the fifth day were undergoing repair. Hence, although autolytic processes were going on in the tissue at that time, the products were removed as fast as they were formed and no increase in amount occurred in the organ.

The same differences in the velocity and degree of autolysis *in vitro* between the washed and unwashed organs are also very evident and require no discussion since they are due to the same causes. It is interesting to note that the nitrogen occurring as monamino-acids reaches at the end of the first day about 80 per cent. of the non-coagulable nitrogen and then although autolysis may greatly increase, their formation increases only in the same proportion. The exception to this is in case of Dog 48, already discussed, where the percentage was high in the tissue itself and remained at the same level during autolysis *in vitro*. This would seem to point to the fact that as far as monamino-acids are concerned their formation in autolysis *intra vitam* occurs in the same manner and by the same chemical processes as they do in autolysis *in vitro*.

v. Drjewecki found that at the end of seventy-two hours autolysis the monamino-acids took up about sixty per cent. of the total nitrogen of the tissue. This figure is somewhat higher than we obtained at this stage of autolysis but in some instances it was reached on the fifth or eighth day.

Ammonia.—As a result of the work of Loewi,¹⁴ Jacoby,¹⁵ Lang and others, considerable interest has become attached to the power of the surviving liver tissue to produce ammonia, especially in view of the current opinion as to the importance of this product, after it has been split off from the amino-acids, as a step in the formation of urea. This interest has been heightened by the appearance of ammonia in increased absolute and percentage amounts in the urine in certain hepatic disorders, thus apparently bringing these matters into correlation.

We have studied the question in two ways. First, in connection with the nitrogen partition of the autolysis now under discussion, and secondly, after the manner of the discoverer¹⁵ of the ammonia-forming power of the liver. This latter part of the work will be discussed separately.

In the partition tables it is seen that although the ammonia content of the necrotic livers is greater than that of the normal it runs parallel with the increase in the amount of non-coagulable nitrogen. Hence the percentage figures show no regular increase or diminution though variations occur owing to the large limit of error dependent on the small amounts of ammonia formed. A comparison of a forty-eight hour necrotic liver (56), which offers an exception to the above statement, in that it shows a progressive diminution with a normal washed liver (53), is instructive. In 53 the normal percentage of ammonia of the non-coagulable nitrogen runs along at about 4 per cent. throughout the experiment. In 56, however, the control shows a high initial ammonia content (10.2), which on the third day dropped to that of the normal washed liver (53) and remained at that level to the end of the experiment.

¹⁴ Loewi, O., Ueber das Harnstoffbildende Ferment der Leber, *Zeit. f. physiol. Chem.*, 1893, xxv, 511.

¹⁵ Jacoby, M., Ueber die fermentative Eiweisspaltung und Ammoniakbildung in der Leber, *Zeit. f. physiol. Chem.*, 1900, xxx, 149.

We explain this variation in the ammonia formation by the assumption that this tissue contained *intra vitam*, as the result of the necrosis, proportionately larger amounts of ammonia liberating compounds than the normal. As, however, the autolysis proceeded less of these products were formed in relation to the non-coagulable nitrogen than was the case in the autolysis of the normal. Hence the percentage figure dropped. Or if we allow for the initial difference we find that the percentage increase is the same in both cases.

The great increase in the amount of non-coagulable nitrogen which occurred between the first and third day (18.5 to 65.1 per cent.) in Dog 56 could not have included the formation of ammonia compounds, since the increase of these latter bodies in relation to the total nitrogen was so slight that there occurred an actual percentage decrease in relation to the non-coagulable nitrogen.

All this would seem to indicate that the production of ammonia which occurs in the autolysis of the liver *in vitro* is the result of a decomposition of coagulable nitrogen in the cell. That is deamidization of the amino-acids and the splitting of urea does not take place to any greater extent in the necrotic tissue undergoing autolysis than it does in the normal.

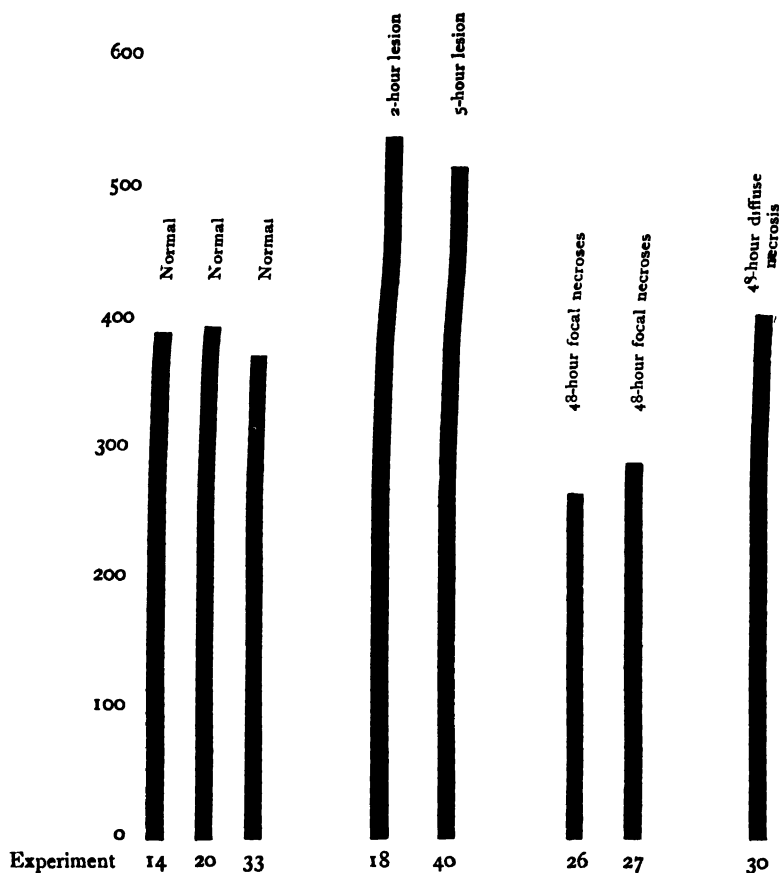
Separate Ammonia Determinations.—These experiments were some of the earlier ones performed and were carried out after the fashion of the investigations reported by Jacoby. A weighed amount, 200 grams, of the finely divided fresh liver tissue was made up to 600 cubic centimeters with distilled water and the well-mixed fluid divided into twelve equal parts. Four of these samples were sterilized immediately for controls. Two were analyzed at once as initial controls, while two, for the purpose of final controls, were placed with the remainder of the portions in the thermostat. At varying periods, duplicate samples were removed for analysis. The ammonia was determined according to a modification of Shaffer's method for the urine. The accompanying diagram indicates in a schematic way the results of the experiments.

It is somewhat difficult to make comparison of these results with those just reported in the partition series since we have no determination of the amount of non-coagulable nitrogen at the various

TABLE II.

Ammonia.

Percentage increase at the end of ten days.



periods; hence the figures represent only the percentage increase of ammonia nitrogen based on its amount in the control sample. Although Jacoby gives in his tables only the amount of ammonia nitrogen formed without taking into consideration the question of dry solids and nitrogen of the dry residue, a recalculation of his figures from average results of our normal livers indicates that more ammonia nitrogen was formed in our experiments than in his. On the other hand, however, our figures are not as high as those re-

ported by Soetbeer.¹⁶ In opposition to the partition series, our figures indicate a larger amount of ammonium compounds in the initial control sample of the normal tissue than in those with varying degrees of necrosis and degeneration. This seeming anomaly we are unable to understand or explain. As, however, the percentage increase figures upon which the diagram is calculated are based upon the initial control as zero, the variable and anomalous control factor is excluded.

Considered in this way it will be seen that the three normals showed an increase of ammonia-nitrogen over the control of from 378 to 396 per cent., an agreement which serves well as a basis for comparison of the experiments on necrotic and degenerated tissue. In the case of the focal necroses the increase is less than the normal, amounting only to 265 to 290 per cent. On the other hand, the diffusely necrotic tissue evidenced the same power to produce ammonia-forming compounds as the normal. In the two samples of congestion and thrombosis, the lesion being of two to five hours' duration, more ammonia was produced than in the normal. These results would seem to indicate that during the initial stage of the process when the liver is intensely congested an increase in ammonia output must occur. This, however, is not supported by our metabolism experiments.¹⁷

Uric Acid.—The investigation concerning uric acid has yielded results of not sufficient interest for presentation in a table. The only point of importance is that a gradual diminution occurs which ceases on the third day. Since in the autolysis of uric acid ammonia is formed, this factor must influence to a slight extent the increase in ammonia observed in the partition experiments.

Arginase.—In the endeavor to explain the results in connection with the hexon bases, reported in the first paper of this series, a few experiments were conducted in the attempt to prepare from normal and necrotic livers an active substance, according to the

¹⁶ Soetbeer, F., Ueber einen Fall von akuten Degeneration des Leberparenchyms, *Arch. f. exper. Path. u. Pharm.*, 1903, 1, 294.

¹⁷ See third paper of this series "Nitrogenous Metabolism" in this number of the *Journal*.

method of Kossel and Dakin,¹⁸ which would hydrolyze arginin into ornithin and urea. Preparations were made by both the ammonium sulphate and acetic acid-ether methods outlined by these investigators and solutions of these were added in aliquot parts to an arginin solution of known strength. The determinations were made sometimes upon the phosphotungstic precipitate, sometimes upon the filtrate from this and once upon both. The aliquots were allowed to autolyze for one, three and five days and controls were done at the beginning and at the end of the experiment.

TABLE III.

Arginase.

Experiment.	Lesion.	Method of Preparation.	Estimation on Phosphotungstate.	c.c. N/10 Acid.			
				Control.	1 day.	3 days.	5 days.
15	Normal.	$\frac{3}{4}$ Saturation $(\text{NH}_4)_2\text{SO}_4$ precipitate.	filtrate.	1.6	2.65	2.75	2.65
15	Normal.	ditto.	filtrate.	4.55	5.95	6.25	6.80
15	Normal.	ditto.	precipitate.	5.75	4.45	4.25	3.65
19	Normal.	ditto.	precipitate.	4.50	3.40	5.00	5.65
42	Normal.	Extraction acetic acid.	filtrate.	5.20	4.45	4.15	4.30
18	2 hours.	Complete saturation $(\text{NH}_4)_2\text{SO}_4$ precipitate.	precipitate.	11.45	12.10	12.40	9.90
40	5 hours.	Extraction acetic acid.	filtrate.	5.40	4.65	5.30	4.40
16	Focal necroses.	Complete saturation $(\text{NH}_4)_2\text{SO}_4$ precipitate.	precipitate.	6.75	6.70	6.55	5.70
28	Diffuse necrosis.	ditto.	precipitate.	6.10	6.90	6.95	6.10
28	Diffuse necrosis.	Extraction dilute HCl.	precipitate.	8.05	9.80	8.45	8.60

Our results in regard to the normal liver agree with those of Kossel and Dakin. The preparations from necrotic livers gave negative or doubtful results, thus affording valuable confirmatory evidence of the position which we assumed as a result of our work upon the hexon bases, namely, that in extreme diffuse necrosis where large areas remote from the circulation are undergoing necrosis

¹⁸ Kossel, A. and Dakin, H. D., Ueber die Arginase, *Zeit. f. physiol. Chem.*, 1904, xli, 321. Weitere Untersuchungen ueber fermentative Harnstoffbildung, *ibid.*, 1904, xlii, 181.

there occurs a marked increase in hexon base content of the tissue. In such areas, evidently, the arginin is not split up to any noticeable extent. The experiments of Wakeman¹⁹ do not show the presence of arginin to the marked extent which the author claims, as we have explained in our discussion of the hexon bases.²⁰

The table shows that of the ten preparations made from seven different livers, three normal, two necrotic and two with thrombosis, only one, the normal (15), showed any activity. In the others either no results were obtained or were so irregular that the experiments may be considered as negative.

In this one experiment with normal liver in which the precipitate obtained by complete saturation of the three-quarter saturated filtrate with ammonium sulphate was employed, the nitrogen of the filtrate from the phosphotungstic precipitate showed a gradual increase presumably due to the autolysis of the arginin added. These results would indicate that an active arginase can be obtained only from normal tissue. This is in complete accord with the results reported in the paper on the hexon bases in which it is shown that during the autolysis of necrotic liver tissue an increase in the hexon base content of the cell occurs.

Leucin and Tyrosin.—In view of the well-recognized presence at times of monamino-acids in the urine of individuals suffering with hepatic disorders, particularly acute yellow atrophy, and of the varying results reported by the different observers in regard to the question of the presence of these compounds in the liver tissue, it seemed advisable to examine the urine and the liver of the animals under observation as to the presence of leucin and tyrosin.

It would appear to be unnecessary for us to enter into a discussion of the older literature concerning the variation in the results as to the presence or absence of leucin and tyrosin in the urine under many pathological conditions. This subject has been well discussed by Ewing and Wolf,²¹ who conclude that the differences observed

¹⁹ Wakeman, A. J., On the Hexon Bases of Liver Tissue under Normal and Certain Pathological Conditions, *Jour. of Exper. Med.*, 1905, vii, 292.

²⁰ See first paper of this series, "Hexon Bases" in this number of the *Journal*.

²¹ Ewing, J. and Wolf, C. G. L., The Clinical Significance of the Urinary Nitrogen, *Amer. Jour. of the Med. Sciences*, 1906, cxxxi, 751.

are most probably due to faulty methods of technique and of confirmation. Of the recent work, to which this criticism cannot properly be applied is that of Taylor,²² who found these monamino-acids present in the liver of acute yellow atrophy as well as in that of probable chloroform poisoning. In both instances, supposedly, necrosis of varying degree had taken place. Wells²³ in a preliminary communication confirms these results for acute yellow atrophy. On the other hand, however, in other conditions to which he gives the general term of "degeneration," Taylor failed to find these substances. Again leucin and tyrosin usually appear in the urine of persons or animals poisoned with phosphorus and this fact has been associated with the occurrence of the well-known hepatic changes, chiefly fatty infiltration, which are known to occur in this condition.

The recent method devised by Fischer and Bergell, in which β -naphthalin sulphochloride is employed, and Abderhalden and Barker's modification of Fischer's esterification method are so time-consuming that we decided that for the purpose in view, the simpler methods were of sufficient accuracy to warrant their use. Ewing and Wolf in the paper mentioned above criticize severely the lead acetate method, originally employed by Frerichs and Städeler. They claim that the microscopic demonstration of leucin and tyrosin by this procedure is unreliable and the crystals supposed to be leucin may be in reality urates or urea. We have used a modification of the lead acetate method in which after the removal of the excess of lead by means of hydrogen sulphide the filtrate is evaporated to dryness and the residue extracted with several portions of absolute alcohol to remove the urea, after which it is treated with repeated portions of ammoniacal absolute alcohol. The united extracts are allowed to evaporate almost to dryness, when characteristic crystals appear, if leucin or tyrosin is present in the original material. When sufficient quantities were present these microscopic findings were controlled by the usual chemical tests. We feel reas-

²² Taylor, A. E., Ueber das Vorkommen von Spaltungsprodukten der Eiweisskörper in der degenerirten Leber, *Zeit. f. physiol. Chem.*, 1902, xxxiv, 580. On the Occurrence of Amino-acids in Degenerated Tissue, *Univ. of California Publications*, 1904, i (Path.), 43.

²³ Wells, H. G., The Composition of the Liver in Acute Yellow Atrophy. Communication read at the first meeting of the Amer. Soc. of Biol. Chemists, Washington, May 8, 1907.

onably sure that the substances upon which we have based the following results were leucin and tyrosin.

TABLE IV.
Leucin and Tyrosin in the Urine.

Experiment.	Lesion.	Leucin.	Tyrosin.	Urine of
2	No necroses	—	+	4th day
34	No necroses	+++	+	1st and 2d day
1	Focal necroses	+	+	1st day
5	Focal necroses	—	+	1st day
23	Focal necroses	++	+	1st and 2d day
32	Diffuse necrosis	—	+	1st day
48	Diffuse necrosis	—	++	1st and 2d day
51	Diffuse necrosis	—	++	2d and 3d day

TABLE V.
Leucin, Tyrosin and Proteoses in the Liver.

Experiment.	Lesion.	Leucin.	Tyrosin.	Proteoses.	Age of Lesion.
16	Focal necroses	—	+	+	48 hour
32	Diffuse necrosis	—	+	+	26 hour
48	Diffuse necrosis	—	++	+	48 hour
51	Diffuse necrosis	—	++	+	48 hour

The table giving the results of the examinations of the urine shows that there is no regularity in the occurrence of these compounds. The type of the lesion has apparently no relation to the amount eliminated and the results presented justify the general consensus of current opinion that the appearance of these compounds is not to be regarded as pathognomonic of any one condition such as acute yellow atrophy or phosphorus or chloroform poisoning. In addition to the positive results presented in the table the urine of nine other animals was examined with negative results. In five of these the liver showed necroses, in four none.

The results of the examination of the liver substance point to the occurrence of tyrosin in larger amounts when the lesion was most pronounced; thus in each of three livers with diffuse necrosis it was present, but in only one of the five examples of focal necroses did it occur. A normal liver and also one with degeneration but no necroses were likewise negative. In no condition did we find leucin. In four livers with extensive necrosis proteoses were found in considerable quantities while a normal liver yielded none. All of

this is in agreement with the variable results of Taylor mentioned elsewhere.

It is evident, therefore, that leucin and tyrosin may be formed during the autolysis of the hepatic tissue, but their appearance in the urine or detection in the liver is dependent upon the condition of the hepatic cells not involved in the lesion. If these cells can take care of large quantities of monamino-acids carried to them normally by the portal vein we see no reason why, if they are present in sufficient numbers and properly functioning, that they should not react in the same way with the same acids formed during the autolysis. The appearance of these monamino-acids under any condition then would depend upon the quantitative relation of the necrosis to the actively functioning cells which are unaffected by the lesion.

Of considerable interest in connection with the finding by Salkowski²⁴ in the urine of various pathological conditions, more particularly a case of yellow atrophy, of an increased amount of nitrogen precipitable by alcohol, is the fact that although in the normal liver the residue remaining after the extraction with absolute alcohol and ammoniacal alcohol is small in amount, in the case of the necrotic tissues this amount is markedly increased. We have examined the residue as to its character and find that it consists mainly of proteoses. The removal of these compounds by way of the blood-stream would cause an increase in the urine of undetermined nitrogen usually ascribed to amino-acids. This occurrence explains those conditions characterized by a high rest-nitrogen without the presence of monamino-acids.

SUMMARY.

1. The presence of blood serum has a decided inhibitory effect on autolysis. Thus in the normal unwashed organs the non-coagulable nitrogen increase was 100 to 300 per cent., while in the washed it amounted to 450 per cent. The washed necrotic livers showed an increase of from 600 to 850 per cent., while that of the unwashed necrotic was only slightly above the normal unwashed.

2. While the initial amount of non-coagulable nitrogen varies it is greater in those livers showing the more extensive forms of

²⁴ Salkowski, E., Zur Kenntnis der Alkoholunlöslichen bzw. kolloidalen Stickstoffsubstanzen im Harn, *Berl. klin. Woch.*, 1905, xlii, 1581, 1618.

necrosis. The final amount of autolysis is also greatest in livers of this type. As regards the rate of autolysis fifty per cent. of the total occurs in the first day in the normal and in all types of lesions both washed and unwashed. The maximum is usually reached on the third day in the unwashed, while in the washed there is a continued increase to the eighth day. At this time in the necrotic livers about two to three times as much of the total nitrogen is in the form of non-coagulable nitrogen as in the normal.

3. In the necrotic tissue the initial controls show the content of monamino-acids, with one exception, to be practically doubled. In the washed necrotic the final amount is seventy per cent. of the total nitrogen against forty-six to fifty-seven per cent. in the washed normal. In all cases the monamino-acid nitrogen runs parallel to the nitrogen in non-coagulable form, but in relation to the total nitrogen it shows a greater increase in the washed than in the unwashed organs.

4. The ammonia production in the necrotic livers as shown by the partition experiments is greater than that in the normal and this increase corresponds to that of the non-coagulable nitrogen. In the experiments concerning the absolute production of ammonia in the presence of serum a greater amount was produced in the two and five hours' lesions than in the normal livers. On the other hand, the forty-eight hour diffuse necrosis equaled the normal and the focal fell below.

5. Arginase was obtained from normal but could not be isolated from necrotic livers.

6. No constant relation could be demonstrated between the anatomical lesion in the liver and the presence of leucin and tyrosin in the urine. Leucin was found occasionally in the urine, but none in the liver. On the other hand, tyrosin was constantly present in livers with diffuse but rarely in those with focal necrosis. In the instances of diffuse necrosis in which the liver and urine of the same animal were examined tyrosin was found in both.

7. The presence of large amounts of proteoses in the necrotic liver indicates that the elimination of these substances (colloidal nitrogen of Salkowski) under such circumstances may account for a part of the total nitrogen of the urine usually attributed to the monamino-acids.

**EXPERIMENTAL LIVER NECROSIS; III. NITROGENOUS
METABOLISM**

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EXPERIMENTAL LIVER NECROSIS; III. NITROGENOUS METABOLISM.¹

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We present here the results of experiments carried out upon dogs in which the general state of the nitrogenous metabolism of the animals was studied before and after the injection of hæmotoxic immune sera. As has been stated elsewhere² the important organic lesion produced by such sera is a hepatic necrosis either focal or diffuse, according to the amount and strength of the serum employed. Such an experimental lesion seemed peculiarly adapted to the study of the function of the liver in general metabolism and of certain peculiar metabolic derangements which have an analogy to those noted in eclampsia, chloroform poisoning, vomiting of pregnancy and acute yellow atrophy. It must be clearly borne in mind, however, that this experimental method of producing hepatic necrosis allows of the infliction upon the liver of a single injury, in point of time, which if not followed by death is rapidly repaired and which is almost without other disturbing factors. Hence metabolic variations may be transient and last but one or two days, and for this reason the resulting disturbance is not comparable to that produced by a continually acting cause with progressive lesion.

The experiments were carried out upon dogs kept in the usual

¹ Conducted under grants from the Rockefeller Institute for Medical Research. Read before the American Association of Pathologists and Bacteriologists, Washington, May 7, 1907. Received for publication July 2, 1907.

² See first paper of this series, "The Hexon Bases," in this number of the *Journal*.

well-ventilated metabolism cages and fed upon a purin-free diet of casein, cracker dust and lard. The amounts of the three dietary constituents varied for the different animals. They were calorifically sufficient and so regulated that the animal finally came into nitrogen equilibrium. The dogs were catheterized at the end of each twenty-four hour period and the urine thus obtained, added to that voided naturally. This was at all times carefully preserved from changes of any kind until the analytic work was completed. To the total twenty-four elimination was added distilled water to make the volume up to 800 cubic centimeters. When hæmoglobinuria or albuminuria caused the appearance of proteid in the urine this was removed by heat and acetic acid, the coagulum being thoroughly boiled out with water and the washings added to the urine.

Upon these twenty-four hour samples the following determinations were made: total nitrogen by the Kjeldahl-Gunning method; ammonia by the Shaffer method; urea by the Mörner-Sjoquist method; uric acid by the Hopkins-Folin method and creatinin by Folin's colorimetric adaptation of Weyl's qualitative test. The difference between the sum of these various factors and the total nitrogen is given as rest or undetermined nitrogen. Kynurenic acid was also looked for qualitatively in order to obtain evidence as to variations in the output of this substance.

The general procedure in these experiments was to place the animal upon nitrogen equilibrium and to conduct control determinations for a period of three days, after which the animal was injected with either normal or toxic sera and the experiment allowed to continue until death ensued or the metabolism had regained its normal level as shown by the control period. It is obvious that in experiments of this character it becomes extremely difficult, if not impossible, owing to variance in the toxicity of the sera and the susceptibility of the animals, to regulate the severity of the lesion, especially as the degree of the latter can be determined only by post-mortem examination. This fact prohibits the production in any two experiments, no matter how carefully planned, of absolutely comparable pathological conditions. This difficulty is brought out by the fact that although each new lot of serum was tested for lethal action on dogs of approximately the same weight and conditions as

those prepared for the experiment, only six out of twelve of the latter survived the first twenty-four period. These six, with a control dog receiving normal serum, constitute the experiments on which this communication is based.

These may be divided into four groups: one injected with normal serum in which no histological change took place; one with a weak toxic serum which caused no necrosis, but an extensive granular and vacuolar degeneration; four with toxic sera causing more or less extensive focal necroses and one with toxic serum producing diffuse necrosis.

TABLE I.
(Dog 18, normal serum.)

Date.	Total Nitrogen.	Urea.*	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Nov. 10	6.51	4.99 76.8	0.556 8.5	0.018 0.28	0.677 10.4	0.269 4.0	
11	6.09	4.81 78.9	0.492 8.1	0.013 0.21	0.633 10.4	0.142 2.4	
12	6.52	5.25 80.5	0.493 7.6	0.020 0.30	0.607 9.3	0.150 2.3	Injected 12 M. Dose 1: 738. Vomited.
13	6.60	5.06 76.6	0.510 7.7	0.017 0.26	0.576 8.7	0.437 6.7	
14	6.06	4.72 77.8	0.482 8.0	0.021 0.34	0.539 8.9	0.298 5.0	

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

Table I shows the result of the injection of normal serum. With this may be compared also Tables VI and VII, in which it is seen that the toxic serum was preceded by an injection of normal serum. In two of these the dose³ of the normal serum was greater than that of any injection of toxic serum. It will be seen that it was practically without effect although in Dog 48 (Table VI) a slight increase in the output of total nitrogen was evident. A consideration of the nitrogen partition indicates, however, that this increase is mainly at the expense of the rest-nitrogen and is to be explained by traces of foreign proteid of the rabbit's serum injected, too small to be removed by the ordinary methods. We possess, there-

³ The figures representing dosage, for example 1:600, indicate that the dose was in the proportion of one cubic centimeter of serum to 600 grams of body weight.

fore, a series of controls upon which to base our conclusions concerning the effect of the toxic sera.

Table II gives the figures obtained as the result of injecting a weak serum,⁴ which caused extensive hepatic degeneration of the granular and vacuolar type but no necrosis.

TABLE II.
(Dog 12, Degeneration, No Necrosis.)

Date.	Total Nitrogen.	Urea.*	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Oct. 15	4.33	3.67 84.5	0.157 3.6	0.006 0.14	0.302 7.0	0.195 4.76	
16	3.77	3.24 85.7	0.131 3.5	0.005 0.13	0.285 7.6	0.109 3.07	
17	3.76	3.29 87.3	0.120 3.1	0.004 0.13	0.284 7.6	0.062 1.87	
18	3.16	2.72 87.1	0.104 3.3	0.004 0.13	0.241 7.6	0.060 1.87	
19	4.23	3.56 84.1	0.090 2.1	0.004 0.09	0.315 75.	0.261 6.21	Injected 4.30 P.M.; weak toxic serum; dose 1:715.
20	3.37	2.66 78.9	0.165 4.9	0.003 0.09	0.288 8.5	0.254 7.66	
21	3.19	2.69 84.3	0.161 5.1	0.006 0.19	0.268 8.4	0.065 2.01	
22	3.02	2.60 86.1	0.122 4.0	0.002 0.06	0.260 8.7	0.036 1.14	Killed.

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

It is seen that a slight but transient rise in the total nitrogen occurred. A small part of this increase is attributable to the foreign proteid injected, with a corresponding rise in the undetermined nitrogen, both in absolute and percentage amounts. The absolute amount of urea nitrogen increased during the first twenty-four hours after injection but not in sufficient quantities to keep pace with the increase in total nitrogen, hence a percentage decrease occurred. The following day both the percentage and absolute amounts diminished markedly to be followed on the succeeding days by return to the normal percentage of urea output. The absolute quantity remained low since the total nitrogen did not return to nor-

⁴ The serum of a rabbit which was not bled until six weeks after immunization against dog's blood. Sera obtained so long after injection frequently show diminished hæmagglutinative and hæmolytic power.

mal. The ammonia output during the twenty-four hours succeeding the injection suffered a decided diminution both in percentage and absolute figures. This decrease was exceedingly transient, since on the succeeding days the reverse occurred and both the absolute and percentage figures went much above the normal. The uric acid and creatinin showed no change but the rest-nitrogen increased considerably on the first and second days following the injection.

The reaction⁶ for kynurenic acid was positive on the day subsequent to the injection and passed off gradually. This would indicate an increase in proteid destruction.⁷

Of most importance is the diminution in the percentage of the total nitrogen eliminated as urea associated with a somewhat corre-

TABLE III.
(Dog 25, Focal Necroses.)

Date.	Total Nitrogen.	Urea.*	Ammonia.	Uric Acid.*	Creatinin.	Undetermined Nitrogen.	Notes.
Dec. 2	4.77	3.55	0.443		0.405	0.372	
		76.5	9.1		8.4	6.0	
3	5.42	4.39	0.507		0.362	0.161	
		81.0	9.4		6.7	3.3	
4	5.24	4.11	0.440		0.298	0.392	
		78.4	8.4		5.7	7.5	
5	4.97	3.86	0.421		0.335	0.354	
		77.6	8.5		6.7	7.2	
6	6.02	4.68	0.496		0.362	0.482	Injection 2:30 P.M. toxic serum dose 1 : 1000.
		77.4	8.2		6.0	8.4	Vomited.
7	5.85	4.77	0.443		0.351	0.286	Hburia.
		81.5	7.6		6.0	4.9	
8	5.92	4.95	0.313		0.323	0.334	Hburia.
		83.6	5.3		5.3	5.8	
9	9.33	7.66	0.587		0.430	0.653	Hburia.
		82.1	6.3		4.6	7.0	
10	6.46	5.40	0.376		0.360	0.324	
		83.5	5.8		5.6	5.1	
11	8.15	7.16	0.404		0.368	0.218	
		87.8	4.9		4.5	2.8	
12	8.22	7.07	0.461		0.366	0.323	No food taken ;
		86.0	5.6		4.5	3.9	killed.

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

⁶ Amounts so small that they were not calculated.

⁷ Treatment of the urine with bromine water.

⁸ Mendel, L. B. and Jackson, H. C., On the Excretion of Kynurenic Acid, *Amer. Jour. of Physiol.*, 1898, ii, 1.

TABLE IV.
(Dog 43, Focal Necroses.)

Date.	Total Nitrogen.	Urea *	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen	Notes.
Feb. 2	7.74	6.31 81.5	0.444 5.7	0.018 0.23	0.417 5.4	0.551 7.2	
3							Fæces mixed with urine.
4	6.95	5.50 79.1	0.406 5.8	0.013 0.17	0.338 4.9	0.693 10.0	
5	6.45	5.37 83.2	0.335 5.2	0.015 0.23	0.280 4.3	0.450 7.1	Injected 10:30 A.M. toxic serum dose 1 : 1738. Vomited.
6	9.91	8.48 85.5	0.478 4.8	0.073 0.73	0.343 3.5	0.836 8.5	Injected 3 P.M. toxic serum; dose 1 : 1200.
7	10.56	8.88 84.1	0.578 5.5	0.045 0.42	0.405 3.8	0.652 6.2	
8	7.65	6.31 82.5	0.479 6.2	0.009 0.12	0.386 5.0	0.466 6.2	
9							Urine lost.
10	4.85	3.84 79.2	0.288 5.9	0.007 0.14	0.341 7.2	0.374 7.6	Killed.

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

sponding increase in the percentage output of ammonia and rest-nitrogen. This is the urinary picture which recently has been described as associated with the hepatic disorder supposed to underlie the symptoms of chloroform poisoning, toxæmia of pregnancy and like conditions and which will be discussed more in detail after our results have been completely given.

Tables III, IV, V, VI and VII present the results of the experiments in which a true necrosis, either focal or diffuse, was obtained.

In these experiments the injection of the toxic sera⁸ was always quickly followed by a more or less marked increase in the elimination of total nitrogen which *persisted* for several days. During the first or second twenty-four hour period, after injection, occurred a slight increase in the percentage of urea nitrogen (three to five per cent.) which was followed on the succeeding days by a drop to normal, and in one experiment below normal. The ammonia nitro-

⁸ In three of these experiments the first injection was followed by a second after a varying interval. This fact renders the figures after the time of the second injection less comparable.

gen percentage of the total nitrogen diminished gradually after the injection and reached its lowest point about the second or third day, after which, in some cases, as in Dogs 49 and 43, it returned to normal; in others, as in Dogs 25, 45 and 48, it remained low. The more advanced the repair at the time of death, the nearer the percentage of ammonia nitrogen had returned to the normal.

The uric acid nitrogen suffered a marked, though transient, increase.⁹ In the three experiments where successive injections were given, the second injection in each instance caused an increase of uric acid on the following day after which it returned to normal.

The absolute creatinin nitrogen output was noticeably augmented after injection; this increase, however, was not quite in the same proportion as the total nitrogen, hence the creatinin nitrogen per cent. of the total tended at times to show a slight diminution.

TABLE V.
(Dog 45, Focal Necroses.)

Date.	Total Nitrogen.	Urea.*	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Feb. 3	6.70	5.50 82.1	0.342 5.1	0.014 0.21	0.274 4.1	0.570 7.5	
4	6.57	5.37 81.7	0.491 7.5	0.013 0.20	0.296 4.5	0.400 6.1	
5	6.43	5.05 78.5	0.452 7.0	0.012 0.18	0.274 4.1	0.642 10.2	
6	6.52	5.33 81.7	0.455 7.0	0.013 0.20	0.265 4.1	0.457 7.0	Injected 10 A. M. toxic serum; dose 1:1500
7	8.99	7.51 83.5	0.560 6.2	0.105 1.17	0.359 4.0	0.456 5.1	
8	7.97	6.25 78.4	0.524 6.6	0.010 0.13	0.300 3.9	0.886 11.0	Injected 10 A. M. toxic serum; dose 1:1000
9	5.30	4.01 75.6	0.441 8.3	0.055 1.04	0.298 5.7	0.928 9.4	
10	10.30	8.20 79.6	0.646 6.3	0.030 0.28	0.349 3.4	1.075 10.4	
11	8.37	6.71 80.2	0.598 7.1	0.021 0.25	0.313 3.7	0.728 8.8	
12	7.45	5.99 80.0	0.447 6.0	0.009 0.12	0.326 4.5	0.678 9.4	Killed

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

⁹ For a detailed discussion of this subject see fourth paper of this series, "Nuclein Metabolism," in this number of the *Journal*.

TABLE VI.
(Dog 48, Diffuse Necrosis.)

Date.	Total Nitrogen	Urea.*	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Feb. 19	6.80	5.90 86.7	0.374 5.5	0.018 0.26	0.373 5.5	0.135 2.0	Injected 5 P. M. normal serum; dose 1:600.
20	6.55	5.53 84.4	0.360 5.5	0.018 0.27	0.364 5.6	0.278 4.2	
21	6.38	5.41 84.8	0.358 5.6	0.024 0.37	0.326 5.2	0.228 4.0	
22	7.00	5.76 82.3	0.406 5.8	0.024 0.34	0.338 4.8	0.472 6.8	
23	6.53	5.50 84.2	0.376 5.8	0.021 0.32	0.326 5.0	0.307 4.7	
24	6.10	5.00 82.0	0.400 6.6	0.018 0.30	0.314 5.1	0.368 6.0	Injected 10 A. M. toxic serum; dose 1:1155. Vomitus mixed with urine.
25				0.036			
26	8.78	7.58 86.3	0.365 4.1	0.018 0.20	0.345 4.0	0.472 5.4	Injected 10 A. M. toxic serum; dose 1:600. Vomited. Hburia. Vomiting; refused food.
27	9.11	6.80 (?) 76.4	0.551 6.0	0.018 0.20	0.349 3.8	1.392 (?) 13.6	
28	11.12	9.24 83.1	0.418 3.8	0.130 1.17			
Mar. 1				0.083			Died.

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

The undetermined or rest-nitrogen which in a general way may be said to indicate the output of amino-acids, polypeptids or proteose-like bodies¹⁰ also underwent a decided increase after injection.

An increase in kynurenic acid elimination after injection was noticed at times, but this was slight at the best and in no way corresponds to the increase which occurs after the administration of phosphorus, phlorhizin and large quantities of meat. In these latter instances Mendel and Jackson¹¹ showed that the increased kynurenic acid output was associated with augmented endogenous

¹⁰ Salkowski, E., Zur Kenntnis der Alkohollunlöslichen bzw. colloidalen Stickstoffsubstanzen im Harn, *Berl. klin. Woch.*, 1905, xlii, 1581, 1618.

¹¹ Mendel, L. B. and Jackson, H. C., On the Excretion of Kynurenic Acid, *Amer. Jour. of Physiol.*, 1898, ii, 1.

or exogenous destruction of proteid material containing the tyrosin nucleus.

As has already been stated, we believe that the effect of the blood changes produced by the serum is directed almost entirely upon the liver and represents a single attack upon this organ. Two coincident conditions, which however do not affect the results in any manner, require perhaps a brief notice. In the first place, vomiting usually occurs and persists for a short time, five to ten minutes, after the injection. The feeding and injection were so arranged that nothing was lost in this manner and as the vomiting also occurred when normal serum was used, with no apparent effect on the metabolism, we believe this factor may be disregarded. It has occasionally, however, caused the loss, on account of admixture of vomitus, of a day's urine.

TABLE VII.
(Dog 49, Focal Necroses.)

Date.	Total Nitrogen.	Urea *	Ammonia.	Uric Acid.	Creatinin.	Undetermined Nitrogen.	Notes.
Feb. 22	5.24	4.33	0.366	0.021	0.215	0.308	
		82.6	7.0	0.40	4.1	5.9	
23	5.48	lost	0.320	0.018	0.218	4.0	
			5.9	0.33			
24	5.46	4.41	0.345	0.021	0.208	0.476	Injected 10 A. M.
		80.8	6.3	0.38	3.8	8.7	normal serum ;
25				0.018			dose 1 : 600
26	5.25	4.34	0.297	0.021	0.194	0.398	Vomit mixed
		82.7	5.7	0.40	3.7	7.5	with urine
27	5.63	4.77	0.320	0.022	0.204	0.314	Trace albumin
		84.8	5.7	0.39	3.6	5.5	Injected 10 A. M.
28	7.07	6.07	0.245	0.014	0.189	0.452	toxic serum; dose
		85.6	3.5	0.20	2.7	8.0	1 : 628. Vomited
Mar. 1	9.14	7.56	0.270	0.029	0.225	1.056	Hburia
		82.7	2.8	0.32	2.5	11.7	
2	8.45	6.98	0.428	0.017	0.221	0.804	Hburia
		82.6	5.1	0.20	2.6	9.5	
3	6.97	6.04	0.370	0.022	0.214	0.324	Hburia
		86.6	5.3	0.31	3.1	4.7	
4	5.70	4.92	0.366	0.020	0.196	0.198	Hburia. Killed.
		86.3	6.4	0.35	3.4	3.6	

* Figures in upper left-hand corner represent grams nitrogen; those in lower right-hand corner, percentage of total nitrogen.

In the second place hæmoglobinuria, sometimes but not always, makes its appearance after twenty-four hours. This condition, however, could not have produced the changes in the general metabolism which we have described since in two of the experiments (43 and 45), where no hæmoglobin or bile appeared in the urine, the results were the same as in those showing a well-marked hæmoglobinuria. This agrees with the observations of Samuely¹² who found in experimental anæmia, produced by means of pyrodin, that the appearance of hæmoglobin or bile in the urine stood in no direct relationship to the changes which took place during the anæmia. On the other hand, Andrea¹³ in a series of experiments, in which various hæmolytic substances (phenylhydrazin, pyrogallol, p-phenylendiamin, glycerin) were administered to rabbits, has found an increase of urea after the initial injection, but a decrease of one third after subsequent injections. The increase he explains by destruction of hæmoglobin and the decrease as due to impaired hepatic function.

Another possibility, however, must also be considered. This is that the temporary anæmia which is simultaneously produced as the result of the primary action of the serum on the red cells may originate changes in oxidization capable of accounting for some of the results. If the anæmia were general in character and of the type which occasions a greatly diminished oxidative power throughout the body, such as is noticed after carbon monoxide poisoning, then we should expect to find, among other disturbances, the elimination of incompletely oxidized products of catabolism, such as lactic acid. We have searched for the appearance of this substance in the urines of five of the animals showing necrosis, but have failed to find it. This, with other facts, appear to justify the exclusion of the factor of diminished oxidation.

In this connection it is of considerable interest to note also that in the type of anæmia produced by Samuely the power of the body to oxidize aromatic compounds, such as phenylalanin and cystein,

¹² Samuely, F., Stoffwechseluntersuchungen bei experimenteller Anämie, *Deut. Arch. f. klin. Med.*, 1907, lxxxix, 220.

¹³ Andrea, P., Influenza della sostanze emolitiche sulle funzioni ureogenetica ed antitossica del fegato, *Arch. Int. de Pharmacodyn. et de Therapie*, 1905, xiv, 389.

was somewhat decreased, but that the metabolism in regard to the fatty amino-acids was absolutely unchanged.

Upon the whole, therefore, it seems justifiable to designate, as the main causative factor in the production of the results obtained, the necrotic lesions more or less diffusely distributed throughout the liver.

Several attempts have been made to study the influence of hepatic necrosis upon the metabolism. Jacoby¹⁴ was the first to study the effect of tying off the vessels supplying certain lobes of the liver. Unfortunately the animals did not survive the operation a sufficient length of time to allow observations upon the urine and he was compelled to content himself with demonstrating that products of autolysis were present in the lobes shut off from the circulation. Doyon and Dufourt¹⁵ report that upon tying off the hepatic artery they obtained a diminished formation of urea and increase in ammonia. Their results were somewhat unsatisfactory, however.

From the clinical side of the question quite recently a considerable amount of data which bears upon the question at hand has accumulated. Many investigators have studied the urinary changes occurring in certain metabolic disorders associated with hepatic diseases. Thus Schittenhelm¹⁶ reports that in chronic diseases of the liver the ammonia output in relation to the total nitrogen elimination is increased. Axisa¹⁷ states that in liver abscesses the same change is associated with a marked decrease in the urea percentage. Ingelrans and Dehons¹⁸ corroborate these findings in hepatic insufficiency and claim that cirrhosis gives the same picture as acute yellow

¹⁴ Jacoby, M., Ueber die fermentative Eiweisspaltung und Ammoniakbildung in der Leber, *Zeit. f. physiol. Chem.*, 1900, xxx, 149.

¹⁵ Doyon, M. and Dufourt, L., Contribution à l'étude de la fonction ureopietique du foie; Effets de la ligature de l'artère hépatique et de celle de la veine porto (*Arch. de physiol. normal et path.*, 1898, S. 5, x, 522), Ref. in *Maly's Jahresbericht f. Thierchemie*, 1898, xxviii, 382.

¹⁶ Schittenhelm, A., Zur Frage der Ammoniakausscheidung im menschlichen Urin, *Deut. Arch. f. klin. Med.*, 1903, lxxvii, 517.

¹⁷ Axisa, E., Ueber Harnstoff und Ammoniakausscheidung im Harn bei Leberabszess, *Zent. f. innere Med.*, 1905, xxvi, 929.

¹⁸ Ingelrans, L., and Dehons, M., La valeur clinique de quelques signes urinaires considérés comme révélateurs de l'insuffisance hépatique, *Arch. de med. exper. et d'anat. path.*, 1903, xv, 188.

atrophy. De Rossi,¹⁹ on the other hand, offers evidence that not all diseases in which lesions of the liver are present show this altered relation of urea to ammonia elimination and concludes that the liver is not the only seat of the formation of urea.

In regard to acute yellow atrophy, vomiting of pregnancy, eclampsia, delayed chloroform poisoning and phosphorus poisoning, recent investigations seem to show that the hepatic lesions, which are found to be present at autopsy in these conditions, are an important causative factor in the disturbance of metabolism. This disturbance shows itself in the urine by a marked diminution in the output of urea and an increase of the ammonia in relation to the total nitrogen elimination. Williams²⁰ assumes that the urinary picture of pernicious vomiting of pregnancy with its high percentage ammonia output is sufficiently definite to render it a valuable aid in determining the question of inducing labor. On the other hand, he contends that in the condition known as eclampsia there is a diminution in the total nitrogen and percentage urea with no very pronounced ammonia variation. Stone²¹ believes that the vomiting of pregnancy is the result of a toxæmia, the lesions of which are primarily an acute degeneration of the liver amounting sometimes to necrosis and resembling in the fatal cases those of acute yellow atrophy. Zweifel's²² researches confirm the opinion that the causative factor in eclampsia is a diminished oxidation which shows itself in the production and elimination of considerable quantities of p-lactic acid. The increased ammonia output is the result of the neutralization of the excess of acids produced and the diminished urea is due to the removal of quantities of ammonia which normally would be synthesized into urea.

¹⁹ De Rossi, S., Sul valore semeiologico dell'urea et dell'ammonica nelle lesioni epatiche, *Riforma Medica*, 1904, xx, 1177.

²⁰ Williams, J. W., Pernicious Vomiting of Pregnancy, *Surgery, Gynecology and Obstetrics*, 1905, i, 41; *Johns Hopkins Hospital Bul.*, 1906, xvii, 71; *Amer. Jour. Med. Sciences*, 1906, cxxxii, 132.

²¹ Stone, W. S., The Toxæmia of Pregnancy, *Amer. Gynecology*, 1903, iii, 518; Some Further Notes on the Toxæmia of Pregnancy, *Med. Record*, 1905, lxxviii, 295.

²² Zweifel, Zur Aufklärung der Eklampsie, *Arch. f. Gyn.*, 1905, lxxvi, 537.

Ewing and Wolf²³ report observations made upon pregnant women from the results of which they conclude that the various conditions of eclampsia, vomiting of pregnancy and yellow atrophy are but different degrees or manifestations of the same disordered process which probably centers itself in the hepatic cells and leads to the deranged elimination of urea and ammonia. A similar disturbance of metabolism associated with necrosis of the liver, has been found by Bevan and Favill²⁴ in fatal chloroform poisoning.

In view of all this it can readily be seen that the concensus of opinion favors the idea that the changes in the percentage elimination of urea and ammonia which are found to occur in these various conditions are but indications of the same functional lesion which centers itself in the hepatic cell. When necrosis of the liver occurs the cells which ordinarily synthesize ammonia into urea are out of function and the ammonia elimination is increased and the urea correspondingly falls.

With a full appreciation of the necessity of caution in transcribing deductions from the results of animal experiments to the explanation of pathological variations in the human organism, we feel that our results render somewhat doubtful the relationship between the hepatic necrosis of the vomiting of pregnancy, for example, and the urinary finding of a high percentage ammonia output. Wolf²⁵ has already justly criticized the conclusions of Williams in this regard and emphasizes the well-known fact that equally high percentages of ammonia are to be found when for any reason, as in inanition, the nitrogen or the calorific value of the diet becomes insufficient for the replacement of the wear and tear of the cell.²⁶ Schittenhelm has shown the influence of diet in this connection by experiments in chronic hepatic diseases where a high ammonia output is present. He noticed that upon increasing the fat of the diet

²³ Ewing, J. and Wolf, C. G. L., The Clinical Significance of the Urinary Nitrogen, II. The Metabolism in the Toxæmia of Pregnancy, *Amer. Jour. of Obstetrics*, 1907, lv, 289.

²⁴ Bevan, A. D., and Favill, H. B., Acid Intoxication and Late Poisonous Effects of Anæsthetics, *Jour. Amer. Med. Assoc.*, 1905, xlv, 691.

²⁵ Wolf, C. G. L., The Chemistry of Toxæmias in Pregnancy, *New York Med. Jour.*, 1906, lxxxiii, 813.

²⁶ Folin, O., Laws Governing the Chemical Composition of Urine, *Amer. Jour. of Physiol.*, 1905, xiii, 66.

a still further increase in the elimination of ammonia occurred and believes that the ammonia offers simply an indication of the lack of normal oxidation or catabolism of the ingested fatty acids. An examination of the results presented by Williams makes it seem very plausible that the diet in his cases is not an unimportant factor in the results. The figures for the total nitrogen indicate that the patients were practically in a state of diminished nutrition even approaching inanition since the amounts fall anywhere between four to eight grams per day, and more important still, the higher the total nitrogen the lower the ammonia and *vice versa* regardless of the severity of the condition. This same criticism can also be applied to the results of Ewing and Wolf. The daily total nitrogen elimination in their experiments is quite as low as that found by Williams, hence the high ammonia can be equally well attributed, in part at least, to similar causes.

The factor of low and insufficient diet was excluded in our experiments since the animals were upon exact equilibrium. We did obtain, however, severe hepatic lesions consisting of localized or diffuse necrotic areas and the ammonia output of our animals never showed more than the merest increase which was exceedingly transient. At this place emphasis must be laid upon the one experiment in which results comparable to, if not as pronounced as those of Williams and of Ewing and Wolf, were obtained. In this instance (see Table II.), however, the histological findings indicated that we were dealing not with a lesion of necrotic character but with an extensive and diffuse degeneration.

On account of the loose use of terms in pathology, this would seem to emphasize that a clear-cut differentiation between degeneration and necrosis²⁷ must be made histologically if we are to correlate the results of chemical studies and histological findings. The pathological condition "degeneration" does not imply autolysis which occurs only in necrosis. It is evident that one may occur without the other and therefore that the chemistry of the cell depends on its functional activity as determined by its physical state. As a reasonable explanation of why a difference in metabo-

²⁷ See first paper of this series, "The Hexon Bases," in this number of the *Journal*.

lism must be expected in the conditions of degeneration as opposed to necrosis we would present the following: In a generalized hepatic degeneration the lesion affects the protoplasm of each and every cell of the whole organ without destruction of the nucleus. This degeneration may set up enzymotic disturbances, secondary in character, which are not connected directly with the actual life processes of the cell and which may readily again return to normal when the abnormal conditions of the cell are removed. Such a differentiation between the actual life processes of the cell and those of a secondary functional character finds best expression in the German words "Baustoffwechsel" and "Betriebsstoffwechsel." When the disturbance of enzymotic equilibrium occurs, if we grant for the sake of argument the unproven hypothesis that the urea formation is the result of enzymotic relations, there would take place an interference in the production of urea from ammonium compounds without an increase in the output of total nitrogen. A simple rearrangement in the partition factors would evidence itself according to which the ammonia would increase as the urea correspondingly diminished. This is exactly the condition found in our experiment with diffuse degeneration.

On the other hand, in necrosis, the individual cell is destroyed and all its functions cease. Autolysis begins in the same way as it does when death supervenes as the result of the removal of the cell from the body. Under such circumstances there occurs a true protoplasmic decomposition from which the cell can never recuperate. Here the nucleus becomes involved as is shown by the histological picture and in the urine by the occurrence of a marked increase in the elimination of uric acid, purin bases²⁸ and phosphorus.

In necrosis, moreover, although many individual cells are dead and have ceased to functionate, there always remain, unless the whole organ becomes necrotic, in contradistinction to the condition of degeneration, many normal cells, ready and capable of assuming in a vicarious manner the function of those already dead. This "factor of safety" in the liver is well demonstrated by the partial extirpation experiments of Ponfick, while the power of other organs

²⁸ For a detailed discussion of this subject see fourth paper of this series, "Nuclein Metabolism," in this number of the *Journal*.

to assume the urea-forming function is shown by the numerous Eck fistula experiments.

In necrosis, therefore, all that is expected as a urinary finding is the appearance of an increase in the total nitrogen output and of the abnormal products of autolysis such as proteoses, polypeptids and amino-acids; and even these latter, in scattered focal necrosis, need not necessarily appear since as very little liver tissue is destroyed the remaining normal cells still possess the power of splitting these substances, formed by cellular digestion, just as they do similar products of intestinal digestion brought to them by the portal²⁹ vein.

Our results substantiate this theoretical expectation. In the experiments with diffuse necrosis a marked and continued augmentation in the total nitrogen and urea elimination occurred as the result of the removal of the products of autolysis. The diminution in the ammonia output may be ascribed naturally to the increase in the proteid catabolism. Finally the increase in undetermined nitrogen is not definitely to be ascribed to the amino-acids ordinarily considered in this connection since we have not found leucin and tyrosin in the urine in amounts which would compare with those found in acute yellow atrophy.³⁰ It is to be considered rather as in the "colloidal" form as described by Salkowski and Mancini.³¹

SUMMARY.

1. In focal and diffuse necroses of the liver due to hæmotoxic sera there occurs an increased elimination of total nitrogen with a corresponding augmented output of urea. The ammonia excretion becomes slightly diminished at first, but later rises somewhat above normal. The undetermined nitrogen is markedly increased.

2. In diffuse degeneration with no necrosis on the other hand only a slightly increased output of total nitrogen is evident. A

²⁹ Freund, E. and Tæpfer, G., Ueber den Abbau des Nahrungseiweisses in der Leber, *Zeit. f. exp. Path.*, 1906, iii, 632.

³⁰ Riess, L., Phosphorvergiftung und Leberatrophie, *Berl. klin. Woch.*, 1905, xlii (Ewald Festnummer 44 a, 54).

³¹ Mancini, S., Studi un nuovo segno per la diagnosi di insufficienza epatica; Contributo allo studio dell'azeta colloidale nelle urine normali e patologiche, (*Arch. di farmocol. speriment.*, 1906) Ref. in *Biochem. Cent.*, 1906, v, 549.

rearrangement of the urea-ammonia proportion occurs in that the ammonia excretion is augmented while the urea elimination is correspondingly diminished. The undetermined nitrogen rises but little.

3. In control experiments with normal serum no effect is produced.

4. These results would appear to indicate that in lesions characterized by uniform degeneration of the liver parenchyma, in contradistinction to necrosis, there occurs no increased nitrogen elimination but merely a disturbance of the urea-forming function of the cell without the appearance in the urine of products of autolysis. On the other hand in necrosis, of even considerable extent, the total-nitrogen is greatly augmented, as is also the rest-nitrogen; while the production of urea, on account of the persistence of normally functioning liver cells, remains relatively unchanged.

This "factor of safety"³² possessed by the liver is, we think, one of the most important results brought out in this investigation and must be given great weight in any consideration of the chemistry of hepatic disturbances.

³² Meltzer, S. J., The Factors of Safety in Animal Structure and Animal Economy, *Jour. of Amer. Med. Assoc.*, 1907, xlviii, 655.

EXPERIMENTAL LIVER NECROSIS; IV. NUCLEIN METABOLISM.¹

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The experiments here detailed were undertaken for the purpose of determining the process or processes intimately concerned in the increased elimination of uric acid which, as has been shown elsewhere,² accompanies the augmented output of total nitrogen when hæmotoxic serum is injected. The fact that the principal lesion produced by the toxic serum is in the liver lends peculiar interest to the problem, in view of the important part which this organ is supposed to play in the formation of endogenous uric acid. The direct object of the experiments was to determine whether the increased elimination of uric acid in dogs under these conditions was the result of the breaking down of nuclear material during the necrosis which follows the injection, or whether it signified simply a diminished oxidative power on the part of the hepatic cell by which the uric acid normally oxidized to simple complexes is eliminated unchanged. Or, more concisely, does an actual increase in the production of uric acid from the nucleic acids of the decomposed nuclei of the necrotic cell occur or is there a simple rearrangement of enzymotic equilibrium by which less uric acid is decomposed than normally.

¹ Conducted under grants from the Rockefeller Institute for Medical Research. Received for publication July 2, 1907.

² See third paper of this series "Nitrogenous Metabolism" in this number of the *Journal*.

Schittenhelm³ has isolated two enzymes from the spleen and liver, one of which causes a hydrolytic splitting off of the amino group of guanin and adenin transforming these bodies thereby into xanthin³ and hypoxanthin respectively; the other an oxydase, xantho-oxidase (Jones), oxidizes the latter compounds into uric acid. Since adenin, guanin, xanthin and hypoxanthin are found in varying amounts in the different nucleic acids which give character to the nucleoproteid of the nucleus we have a fairly definite process by which uric acid results, it may be assumed, from the decomposition of the nucleus by means of autolytic enzymes aided perhaps by a special oxydase.

On the other hand, however, the fact has long been known that various tissues are capable of decomposing uric acid. Wiener,⁴ Schittenhelm⁵ and Almagia⁶ have recently studied the question in detail and describe the presence of uricolytic enzymes in various tissues of several animals. Schittenhelm claims that the calf's kidney is most active in this regard with the liver of the same animal second in power. Pfeiffer⁷ was able to prove that ninety to ninety-five per cent. of the uric acid added to human kidneys suffered decomposition. In the horse Almagia found the most active enzyme to be in the liver. These results indicate that the liver among other organs possesses the power of forming uric acid by oxidation and perhaps also by synthesis,⁸ while at the same time it is

³ Schittenhelm, A., Ueber die Harnsäurebildung in Gewebsauszügen, *Zeit. f. physiol. Chem.*, 1904, xlii, 251; Ueber die Harnsäurebildung und die Harnsäurezersetzung in dem Auszügen der Rinderorgane, *ibid.*, 1905, xlv, 121.

⁴ Wiener, H., Ueber Zersetzung und Bildung der Harnsäure im Thierkörper, *Arch. f. exper. Path. u. Pharm.*, 1899, xlii, 375.

⁵ Schittenhelm, A., Ueber das uricolytische Ferment, *Zeit. f. physiol. Chem.*, 1905, xlv, 161.

⁶ Almagia, M., Zur Lehre vom Harnsäurestoffwechsel, Ueber die Zersetzung der Harnsäure durch die Organe des Säugetheirs, *Beit. z. chem. Phys. u. Path.*, 1905, vii, 459.

⁷ Pfeiffer, W., Zur Lehre vom Harnsäurestoffwechsel, Ueber die Zersetzung der Harnsäure durch menschliche Nierengewebe, *Beiträge z. chem. Phys. u. Path.*, 1905, vii, 463.

⁸ Burian, R., Ueber die oxydative und die vermeintliche synthetische Bildung von Harnsäure im Rinderleber Auszug, *Zeit. f. physiol. Chem.*, 1905, xliii, 497; Die Herkunft der endogenen Harnpurine bei Mensch und Säugethier, *ibid.*, 1905, xliii, 532.

capable of decomposing it through the stages of allantoin and glyoxylic acid.

Austin⁹ has raised the point, however, that the alkali in which the uric acid is dissolved is capable of splitting up the latter and that all preparations of uricolytic enzymes contain purin bases which readily become transformed into uric acid. Notwithstanding the latter criticism it seems fairly well founded that the reaction under discussion is a reversible one in which synthetic and analytic processes come into a state of equilibrium in the cell and that alterations in the activity of one or the other set of enzymes increase or diminish the elimination of uric acid without the occurrence, necessarily, of an increase or decrease in the amount of purin material formed from the nucleic acid of the nucleus.

If the increased elimination of uric acid in dogs injected with toxic sera is the result of altered uric acid equilibrium of the cell, as just described, one would expect to find that, as more xanthin the hypoxanthin are oxidized to uric acid, less of these purins should be eliminated in the urine. On the other hand, if the uric acid is the result of a new formation from the nucleic acids then an increase in purins also would be expected and at the same time an augmentation in elimination of phosphoric acid in some form¹⁰ as the result of the splitting of the nucleic acid molecule. In the formation of uric acid by the first process no change in phosphoric metabolism should take place.

Methods.—The experiments presented in Tables I, II and III were carried out in the following manner: The animals were placed upon nitrogenous equilibrium after which estimations were made during a three day fore period. An injection was then given, and in two instances a second injection after a lapse of one and three days respectively. The observations were continued until after the maximum effect was reached. The purin-free diet was a casein,

⁹ Austin, A. E., The Uricolytic Enzyme in Animal Organs, *Jour. of Med. Research*, 1906, xv, 309. The Uricolytic Enzyme, *ibid.*, 1907, xvi, 7.

¹⁰ Jackson, H. C. and Blackfan, K. D., Action of Certain Drugs on the Elimination of Uric Acid During a Nitrogen-free Diet, *Albany Medical Annals*, 1907, xviii, 24.

cracker dust and lard mixture. The urine was examined for total-nitrogen by the Kjeldahl-Gunning method, the uric acid and purin bases were determined by the Salkowski procedure, and the inorganic phosphates by titration with uranium nitrate, using potassium ferrocyanide as an indicator. The method used for total phosphorus was fusion of the evaporated residue of the urine with sodium hydroxide and potassium nitrate, precipitation with ammonium molybdate in the presence of ammonium nitrate, solution of the ammonio-phosphomolybdate in ammonia and re-precipitation with magnesium mixture. This precipitate was filtered off, incinerated and weighed as magnesium pyrophosphate.

The question of the presence of phosphorus in the urine in organic form has recently received attention from various investigators among whom Bergmann¹¹ and LeClerc and Cook¹² incline to the opinion that all of the phosphorus of the urine exists in the inorganic form. The latter observers studied the phosphorus content of the urine by means of determinations made by Neumann's¹³ method, and by that outlined above, and compared the results with those obtained by the uranium-titration procedure. They show that the results by the dry fusion method are uniformly higher by three to four per cent. than those given either by uranium acetate or by Neumann's method; hence they incline to the view that this difference is within the limits of error and that no organic phosphorus is present in normal urine.

We cannot agree with these conclusions in view of the results obtained by one of us in a study of the elimination of organic phosphorus after the administration of sodium salicylate.¹⁴ Nor do they receive support from the results which are presented in this

¹¹ Bergmann, W., Ueber die Ausscheidung der Phosphorsäure beim Fleisch- und Pflanzenfresser, *Arch. f. exper. Path. u. Pharm.*, 1901, xlvii, 77.

¹² LeClerc, J. A., and Cook, F. C., Metabolism Experiments with Organic and Inorganic Phosphorus, *Jour. of Biol. Chem.*, 1906, ii, 203.

¹³ Neumann, A., Einfache Veraschungsmethode (Säuregemisch-Veraschung), *Zeit. f. physiol. Chem.*, 1902, xxxvii, 115.

¹⁴ Jackson, H. C. and Blackfan, K. D., Action of Certain Drugs on the Elimination of Uric Acid During a Nitrogen-free Diet, *Albany Medical Annals*, 1907, xviii, 24.

communication. The constant uniformity of difference shown by the fusion method over that of titration seems to indicate that the difference is not due to the factor of error in the method. We have performed in this connection some preliminary experiments with a view to explaining the differences reported and expect to continue them more in detail. At present we can say that Neumann's method apparently yields results which agree closely with those obtained by titration with uranium nitrate, using potassium ferrocyanide as indicator. When the fusion method is employed the figures obtained are uniformly higher than by both of the other methods. At present, therefore, we incline to the opinion that phosphorus in other than inorganic form is present in normal urine.

TABLE I.
Nuclein Metabolism. Dog 59.

Date.	Grams Nitrogen as			Grams P_2O_5 .		Notes.
	Total.	Uric Acid.	Purin Bases	Inorganic.	Organic	
Apr. 10	7.86	0.0015	0.001	0.899	0.147	
11	5.51	0.0159	Lost	0.652	0.093	
12	4.70	0.0141	0.0082	0.690	0.087	
13	4.49	0.0121	0.0097	0.576	0.050	Injection to A. M. toxic serum; dose 1:785.
14	6.97	0.0126	0.0231	1.009	0.085	
15	12.50	0.0246	0.0169	0.690	0.121	Injection 11 A. M. toxic serum; dose 1:1745.
16	11.08	0.0127	Lost	0.848	0.072	Hburia.
17	13.81	0.0319	0.0287	0.629	0.083	
18	11.93	0.0203	0.0265	0.866	0.023	Killed.

TABLE II.
Nuclein Metabolism. Dog 61.

Date.	Grams Nitrogen as			Grams P_2O_5 .		Notes.
	Total.	Uric Acid.	Purin Bases.	Inorganic.	Organic.	
Apr. 22	3.33	0.0042	0.0086	0.478	0.078	
23	3.83	0.0063	0.0072	0.554	0.052	
24	3.12	0.0050	0.0079	0.584	0.097	Injected 12 M. toxic serum; dose 1:937; vomited.
25	4.57	0.0167	0.0103	0.549	0.085	Hburia.
26	5.74	0.0336	0.0107	0.387	0.074	Hburia.
27	6.83	0.0241	0.0124	0.387	0.084	
28	6.54	0.0200	0.0235	0.326	0.046	
29	5.69	0.0112	0.0086	0.356	0.118	Killed.

TABLE III.
Nuclein Metabolism. Dog. 63.

Date.	Grams Nitrogen as			Grams P ₂ O ₅ .		Notes.
	Total.	Uric Acid.	Purin Bases.	Inorganic.	Organic.	
May 14	4.74	0.0057	0.0061	0.520	0.062	Injected 11 A. M. toxic serum; dose 1 : 835; vomited.
15	4.41	0.0054	0.0068	0.557	0.109	
16	4.26	0.0068	0.0076	0.550	0.072	
17	5.29	0.0249	0.0110	0.641	0.062	Injected 11 A. M. toxic serum; dose 1 : 557; vomited. Hburia. Hburia. Hburia. Killed.
18	4.24	0.0143	0.0088	0.508	0.078	
19	4.34	0.0109	Lost.	0.535	0.066	
20	4.75	0.0133	0.0181	0.576	0.066	
21	7.64	0.0141	0.0116	0.435	0.090	
22	8.38	0.0095	0.0187	0.440	0.075	
23	6.21	Lost.	0.0133	0.550	0.111	

Results.—From the figures in the above tables it is evident that the increase of uric acid after injection, as noted in the study of the nitrogenous metabolism,¹⁵ is constant and that there also occurs an increase of purin bases and with one exception of phosphorus pentoxide. This general increase occurs not only after the first but also after subsequent injections. It reaches its maximum on the second day and then falls away. The uric acid increase is greater proportionately than that of the total nitrogen, hence an augmentation of the percentage uric acid in terms of total-nitrogen takes place. The uric acid nitrogen, however, returns to the normal sooner than the latter. The same facts hold true for the purin bases the elimination of which runs parallel, in a general way, to the uric acid output. In the one instance (Table II, Dog 61) in which the phosphorus pentoxide elimination remained constant there still occurred the markedly increased uric acid and purin base output. That the phosphorus pentoxide elimination did not follow that of the uric acid and purin bases is to be explained by the fact that after the injection the animal ate only a quarter of the daily food allowance. Although on the day following the injection the

¹⁵ See third paper of this series "Nitrogenous Metabolism" in this number of the *Journal*.

amount ingested of phosphorus pentoxide was therefore diminished, the elimination of inorganic phosphates remained unchanged. This would indicate that an actual increase in endogenous phosphorus pentoxide formation took place, thus bringing the apparent exception in agreement with our other observations. The organic phosphorus elimination was unaltered.

The question as to the cause of the increased output of uric acid during a purin-free diet, observed after various experimental procedures, has been variously explained. Beebe¹⁸ concludes that the increase found after alcohol administration is to be attributed to a diminished oxidative power on the part of the hepatic cells although the purin bases were likewise increased. In commenting upon this view Jackson and Blackfan, from results which they obtained with alcohol, inclined to the opinion that the facts would warrant rather the assumption that the alcohol causes an increased new formation of uric acid and purin bases, and they adduced as evidence, among other factors, the augmented excretion of organic phosphorus.

According to the view of Schittenhelm, concerning the probable way in which the uric acid is formed from the nucleo-proteid, we would expect, if for any cause oxidization in the hepatic cell is diminished, the xantho-oxydase would be affected, and as a result less uric acid would be formed from the purin bases than under normal circumstances. An increase in purins would also occur if the hydrolytic splitting enzymes were unaffected. An augmentation of uric acid would take place either as the result of a diminished power of the uricolytic enzymes or as the result of an increased new production, other things being equal. In the latter case the purin would also be increased.

The results reported in the paper on nitrogenous metabolism do not indicate that the injection of a hæmotoxic serum causes a prolonged or decided decrease in the oxidative power of the hepatic cell. As a whole the organ continues to perform its functions in the normal way. We must therefore explain the increase in purins and uric acid as a decomposition of nuclear material in the autolysis occurring in the areas of necrosis. This is brought about by

¹⁸ Beebe, S. P., The Effect of Alcohol and Alcoholic Fluids upon the Excretion of Uric Acid in Man, *Amer. Jour. of Physiol.*, 1904, xii, 13.

the hydrolysis of the nucleic acids. Such an explanation agrees with the feeding experiments performed by Sweet and Levene¹⁷ upon a dog with an Eck fistula. They found the ingestion of nucleic acids to be followed by a rise in uric acid and total phosphorus elimination. The fact that the increased excretion of these nuclear compounds usually reaches its maximum in our experiments on the second day, when the autolysis is at its height, would also strengthen this explanation.

The increased elimination of inorganic phosphates which accompanies the output of uric acid would likewise point to the new formation of the latter from the nucleic acids formed as a step in the autolysis of nuclear material. The phosphoric acid radical of these acids is evidently excreted in inorganic form and thus the mechanism differs from that observed in connection with the administration of alcohol and salicylic acid under which circumstances the phosphoric acid is apparently eliminated in organic combination, since no increase in inorganic phosphates occurs.

Allantoïn.—In the dog, the largest part of the quantity of uric acid ingested, as shown by Swain,¹⁸ disappears and is not excreted. In the experiments outlined above it is not improbable, therefore, that a much larger amount of uric acid was produced in the necrosis of the cell nucleus than was eliminated. Of the total amount formed only a small proportion escapes hydrolysis and appears in the urine. Swain also has shown that the ingestion of large amounts of uric acid is followed by the elimination of small quantities of allantoïn.

In order to obtain evidence upon this point the urine of the six animals under observation in the experiments reported in the paper on nitrogenous metabolism, was examined for allantoïn according to the method of Loewi.¹⁹ The results are not uniform but indicate that usually the increase of uric acid is accompanied by an increase in allantoïn; for example, in one the amount of uric acid the day

¹⁷ Sweet, J. E., and Levene, P. A., Nuclein Metabolism in a Dog with Eck's Fistula, *Jour. of Exper. Med.*, 1907, ix, 229.

¹⁸ Swain, R. E., Formation of Allantoïn from Uric Acid, *Amer. Jour. of Physiol.*, 1901, vi, 38.

¹⁹ Loewi, O., Beiträge zur Kenntnis des Nucleinstoffwechsels, *Arch. f. exp. Path. u. Pharm.*, 1900, xlv, 20.

before injection was 0.018 gram and of allantoin 0.319; in the urine of the twenty-four hour period following the former rose to 0.337 and the latter to 0.669.

CONCLUSIONS.

In necrosis of the liver of the dog produced by hæmotoxic immune sera, the increased excretion of uric acid, purin bases and inorganic phosphorus pentoxide is the result of the hydrolysis of nuclear material occurring during the autolysis of the necrotic tissue.

EXPERIMENTAL LIVER NECROSIS; V. THE FATS
AND LIPOIDS

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EXPERIMENTAL LIVER NECROSIS. V. THE FATS AND LIPOIDS.¹

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The results outlined in this communication constitute a partial report of a somewhat comprehensive investigation² now in progress of the chemical processes concerned in the variations occurring in the amounts of fats and lipoids in the hepatic cell under normal and various pathological conditions.

It has seemed advisable, in connection with the other investigations of liver necrosis here presented, to discuss at this time only that part of the general study which deals with the fatty changes in hepatic necrosis brought about by the injection of hæmotoxic immune serum.

The study of such lesions is of peculiar value in view of the attempts which have been made to bring into a relation of cause and effect the autolysis of the organ and the appearance of the fat. We have therefore with this point in view attempted to determine whether in the necrosis (autolysis) which follows the injection of the serum there occurs any alteration in the fat content correspond-

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²This investigation, including a study of the fatty changes occurring in various experimental lesions of the liver of the dog and of certain pathological conditions of the human liver, will be published later in full by H. C. Jackson and L. K. Baldauf. We wish to express here our indebtedness to Dr. Baldauf for the privilege of utilizing in this partial report that portion of his work which refers to necroses of the liver produced by hæmotoxic serum.

ing to changes observed in the nitrogenous constituents of the cell. Especial interest is attached to this question in view of the somewhat widely divergent opinions held as to the origin of the fat which appears in the so-called fatty transformation of various organs. That the fat does not arise from a peculiar decomposition of the proteid molecule in the cell, the fatty degeneration of Virchow, seems fairly well established. On the other hand, Rosenfeld³ and others hold that the fat makes its appearance as a simple infiltration from without when for any reason the cell has received an injury which seems to inhibit its oxidizing power. The appearance of fat in organs during phlorhizin poisoning is thus explained by Lusk.⁴ Waldvogel,⁵ however, who has investigated this question most thoroughly believes that the process is one closely allied to autolysis. His theory is that normally certain substances, which may be called combined fats, such as the ovovitellin of Hoppe-Seyler, or the lecithalbumin of Liebermann, hold the fatty radical in a combination which does not react to microchemical fat stains, such as Scharlach R or Sudan III, and which cannot be removed chemically by the ordinary fat solvents. These substances, however, during autolysis become split in such a manner that the fat radical is liberated in the form of protagon, jecorin, lecithin and even neutral fats. He supports this contention by experiments upon the livers of phosphorus-poisoned animals and upon normal livers undergoing autolysis, in which he has shown there is a marked augmentation during autolysis of such substances as protagon, jecorin, cholesterin, fatty acids and neutral fats. The lecithin, on the other hand, is diminished.

³ Rosenfeld, G., Fetbildung, *Ergebnisse der Physiologie*, 1902, i, 651; *ibid.*, 1903, ii, 50.

⁴ Ray, W. E., McDermott, T. S. and Lusk, G., On Metabolism During a Combination of Phosphorus Poisoning and Phlorhizin Diabetes, *Amer. Jour. of Physiol.*, 1900, iii, 139.

⁵ Waldvogel, Autolyse und fettige Degeneration, *Virchow's Arch.*, 1904, clxxvii, 1. Phosphorvergiftung und Autolyse, *Deut. Arch. f. klin. Med.*, 1905, lxxxii, 437. Waldvogel and Mette, Die Autolyse in Menschlichen fettigen degenerierten Organen, *Münch. med. Woch.*, 1906, ix, 402. Waldvogel, Die durch Fermente bewirkten Umwandlungen bei der fettigen Degeneration, *Zeit. f. physiol. Chem.*, 1904, xlii, 200.

Siebert⁶ has also shown that, although in autolysis the ether extract of the liver does not increase, a marked rearrangement of the fatty compounds of the extract takes place, and that the jecorin rapidly suffers decomposition. This is not in accord with Waldvogel's results. In this connection it may be mentioned that Taylor⁷ has conducted experiments upon normal and phosphorus-poisoned frogs and finds that, although the absolute amount of free fat may not increase after the administration of phosphorus the combined fats estimated after digestion with pepsin-hydrochloric-acid suffer a marked diminution equivalent to two thirds of the amount originally present.

As stated in previous papers in this series phosphorus poisoning does not seem to set up in the liver processes which are strictly analogous to autolysis following necrosis, hence the results to be presented must not be considered as strictly comparable to those obtained in other lesions.

Few attempts have been made to study the effect of a true necrosis upon the fat constituents of the cell. Dietrich,⁸ as the result of histological studies, claims that autolysis is not an important factor, since tissues introduced into the peritoneal cavity in collodion sacs do not show fatty change. When the tissue, however, is not enclosed in sacs, fat droplets appear which are present not in the cell substance but in the interstices of the tissue. He also tied off the renal arteries and found a "deposition" of fat around the necrotic areas. He believes, therefore, that fat will not appear if the cell is completely dead as in necrosis; but only when it continues to functionate incompletely, as, for example, must be the case with the cells around necrotic areas.

This opinion we can confirm as the result of the histological study of focal necroses caused by hæmotoxic serum. Frozen sections of formalin-hardened material stained with Scharlach R never show more fat, and usually less, than the surrounding adjacent

⁶ Siebert, F., Das Verhalten des Fettes bei der Autolyse der Leber, *Beiträge zur chem. Physiol. u. Path.*, 1902, i, 114.

⁷ Taylor, A. E., On Fatty Degeneration, *Jour. of Med. Research*, 1903, ix, 59.

⁸ Dietrich, A., Experimente zur Frage der fettigen Degeneration *Münch. med. Woch.*, 1904, li, 1510.

normal liver tissue. There is always present, however, in lesions twenty-four to forty-eight hours old a very definite and striking accumulation of fat in the ring of more or less degenerated cells lying between the necrotic and normal liver. These cells correspond to those which in hæmatoxylin and eosin preparations present a vacuolated, granular protoplasm and pycnotic, poorly staining, nuclei.⁹

Di Cristina,¹⁰ who conducted experiments somewhat after the nature of those of Dietrich, and made chemical analyses by Rosenfeld's method, states that no increase of fat occurs in the necrosis caused by shutting off completely the renal circulation. Of considerable interest in connection with Dietrich's view are the experiments reported by Bainbridge and Leathes.¹¹ These investigators ligated the hepatic artery alone and thereby obtained an increase in fat but no necrosis. The ligation of the portal vein on the other hand resulted in atrophy of the cells and some necrosis, but no augmentation of fat. These experiments appear to confirm the idea that the cells must retain in part their normal function and be normally bathed with the circulating fluids in order to give rise to the appearance of fat within them.

In concluding this brief discussion which merely suffices to indicate the trend of opinion in regard to the subject under investigation it may be said that much of the discrepancy in the results reported can be safely ascribed to the varying methods employed especially in connection with the extraction of the fatty material from the tissue. Siegert has also emphasized the ease with which the extracted products undergo laboratory changes.

Methods.—The organs were removed from the body quickly, put through a hashing machine, weighed, and dried under absolute alcohol at about 70–80° C. At this stage the partially dry material was weighed and then ground in a machine to an impalpable powder. Part of this was further dried in a desiccator to constant

* Pearce, R. M., Regenerative Changes in the Liver: A Study of Experimental Lesions in the Dog, *Jour. of Med. Research*, 1906, xv, 99.

¹⁰ Di Cristina, Die chemischen Veränderungen bei fer fettigen Degeneration in Beziehung zur den anatomischen, *Virchow's Arch.*, 1905, clxxxi, 509.

¹¹ Bainbridge, F. A. and Leathes, J. B., The Effect of Arterial or Venous Obstruction upon the Nutrition of the Liver Cells, *Biochem. Jour.*, 1906, ii, 25.

weight and upon this was calculated the dry substance and nitrogen content of the original tissue. Another weighed part was extracted in a Soxhlet with alcohol and chloroform successively according to the method of Rosenfeld. Each total extraction with alcohol and with chloroform lasted on an average thirty hours. In some cases the original partly-dried material was so fatty that a rough extraction with chloroform at room temperature preceded the grinding. This extract was added to the subsequent one obtained from the Soxhlet. The total fat was taken up in a definite volume of chloroform. An aliquot portion of this was evaporated to complete dryness at about 70° C. and from this was calculated the fat per cent. of the tissue.¹²

For our purposes it was not thought necessary to keep separate for analysis the alcohol and chloroform extracts as Waldvogel does with alcohol and ether. This procedure is exceedingly time consuming and we have employed another and simpler method which we believe has given results equally definite. Instead of attempting to decide whether the fat compounds present in the extract underwent any change or rearrangement, such as is described by Waldvogel during autolysis of normal tissues and in phosphorus poisoning, it seemed sufficient to determine the nitrogen and phosphorus pentoxide content of the extract, and from these figures to calculate the relationship of the nitrogen to the phosphorus. Since the molecule of the lecithins contains one nitrogen and one phosphorus atom the relationship $P:N = 1:1$; in jecorin, however, this ratio is $1:4$ and in protagon about the same, varying from $1:3.4$ to 4.8 . The latter figure is calculated from the analyses of Dunham.¹³ It is seen, therefore, that the greater the preponderance of substances of the jecorin and protagon type in the extract the higher would be the $P:N$ ratio. On the other hand, if these substances should undergo an autolytic change whereby lecithin and fatty acids were

¹² We have employed chloroform in this connection, since, in the first place, it is a much readier solvent for the fatty compounds than either sulphuric or petroleum ether, and secondly, because all of the ether we could obtain reacted distinctly acid to phenolphthalein, a fact already alluded to by Baldauf, *Chemistry of Atheroma and Calcification (Aorta)*, *Jour. of Med. Research*, 1906, xv, 355.

¹³ Dunham, E. K., Further Observations on the Phosphorized Fats in Extracts of the Kidney, *Proc. of Soc. for Exper. Biol. and Med.*, 1905, ii, 63.

produced, this ratio should fall to the neighborhood of 1 : 1. Hence from variations in this ratio, changes in the fatty constituents of the extract should be readily determined.

Measured portions of the chloroform extract were analyzed in duplicate as follows:¹⁴

Total nitrogen by the Kjeldahl-Gunning method; phosphorus pentoxide by the usual fusion procedure and weighing as magnesium pyrophosphate and the iodine equivalent as outlined by the Association of Official Agricultural Chemists.¹⁵ With some exceptions portions of the tissues analyzed were stained with hæmatoxylin and with Scharlach R for the purpose of determining roughly in a comparative way the extent of the necrotic lesion and the fat content.

Results.—The table presents the results obtained in the analysis of four dog livers with normal, four to five, per cent. fat content, one apparently normal but very fatty liver with 21.9 per cent., five with focal necrosis and three with diffuse necrosis of varying degree. As can be seen no relation exists between the degree of necrosis and the amount of fat present. The high amount of fatty material which is present in the normal liver, extractable by the newer method of Rosenfeld, is at first glance surprising, but is in accord with the results of recent investigators. The fat per cent. of all the necrotic livers falls between the normal limits with the exception of 43 and 21 which are above the normal, but these do not represent the most extensive necrosis. Experiments 28 and 29, with the most diffuse necrosis, show normal amounts of fat.

The point which was emphasized in one of the previous papers¹⁶ concerning the percentage of dry substance in the fatty livers is well shown in the table. Whenever the fat per cent. rose above normal the per cent. of dry substance rose almost proportionately. This relation is readily seen by referring to that column in the table in which is given the per cent. of fat-free dry substance. With two exceptions, the dry substance without fat falls between 18.8 to

¹⁴ In most instances the chloroform had to be *completely* removed before the commencement of the analysis.

¹⁵ U. S. Dept. of Agriculture, 1899, Bulletin 46, 50.

¹⁶ See first paper of this series, "The Hexon Bases" in this number of the *Journal*.

TABLE I.
Fats and Lipoids.

Experiment.	Tissue.						Alcohol-chloroform Extract			Schach R. Fat by	Lesion.	
	Per Cent.						Per Cent.					
	Dry Substance	Dry Substance, Fat-free.	Fat.		Nitrogen.		Nitrogen.	P ₂ O ₅ .	P:N			Iodine Equiva- lent.
			Moist Substance.	Dry Substance.	Dry Substance.	Dry Substance, Fat-free.						
1	25.6	22.6	3.97	15.5	12.7	15.0	—	—	—	58.3	+	Normal.
5	28.6	24.1	4.55	15.9	9.7	11.5	1.8	2.6	1:7.0	60.0	?	Normal.
2	24.5	19.5	4.95	20.2	12.7	15.9	2.5	5.2	1:4.9	56.6	+	Normal.
4	18.5	13.7	4.75	25.7	9.2	11.3	1.5	3.6	1:4.4	53.4	+	Normal.
18	40.7	18.8	21.85	53.7	7.5	12.6	0.6	1.3	1:4.5	47.0	++	Normal (very fatty).
7	29.8	26.7	3.10	10.4	9.3	13.2	3.1	7.3	1:4.3	67.7	?	Focal necroses.
6	25.3	21.2	4.12	16.3	12.5	16.7	2.4	7.0	1:3.5	58.3	?	Focal necroses.
26	28.3	24.5	3.84	13.6	9.9	13.8	2.1	5.0	1:4.3	58.7	+	Extensive focal necroses.
43	41.1	24.9	16.23	39.5	8.3	14.0	0.7	2.0	1:3.8	47.0	++	Extensive focal necroses.
27	26.0	20.3	5.72	22.0	11.5	15.5	1.5	6.5	1:2.4	49.3	++	Extensive focal necroses.
21	41.1	14.9	25.15	61.2	6.7	10.5	0.3	1.1	1:3.0	50.0	++	Diffuse necrosis.
28	23.2	18.8	4.43	19.1	11.9	15.5	2.3	5.8	1:4.3	50.3	++	Diffuse necrosis
29	21.9	20.1	4.88	19.5	12.7	16.3	2.3	6.1	1:4.0	62.3	+	Diffuse necrosis (most marked,

26.7 per cent. and this surprisingly small variation is in no definite relation to the amount of fat present. The two exceptions are one with normal and one with high fat content. Waldvogel has claimed that as the fatty autolysis increases the water content of the tissue also rises. We would be inclined to ascribe this rather to the nitrogenous autolysis *in vivo* which as we have pointed out elsewhere¹⁶ tends to diminish the amount of dry substance if the circulation is not too greatly impaired. This is shown clearly in Experiments 27, 21, 28 and 29, with the most pronounced necrosis, in which it is seen that the figures for the dry fat-free substance lie on the lower edge of the variation limit for this factor.

As regards the nitrogen content of the tissues a somewhat similar condition of affairs is evident, and although this point does not come directly into this part of the general subject it is of considerable interest. It will be seen that the per cent. nitrogen of the fat-containing dry substance varies within rather wide limits (6.7 to 12.7 per cent.). In those instances, however, where the fat content is high the nitrogen per cent. is low, as would be expected. If, however, the nitrogen of the fat-free dry substance is considered, it is found that the figures for this factor are surprisingly constant throughout (10.5 to 16.7 per cent.). Some of the lowest and highest values occur where, as regards the fat content, the liver tissue was perfectly normal. This indicates apparently that in conditions such as those under discussion, when an increase in fat-content occurs, the material which is represented by the nitrogen of the tissues suffers no decrease or increase in amount. If then the fat originates from some compound proteid antecedent, the proteid component remains apparently unchanged.

A consideration of the character of the fatty extract obtained from normal and necrotic tissues presents some interesting facts. In regard to the iodine equivalent, which indicates roughly the content of oleic acid in the fat mixture, it is evident that as the fat per cent. increases the iodine absorption factor falls below normal (Experiments 18, 43, 27 and 21). This low iodine factor associated with increased fat content would of course point to a diminishing content of oleic acid radicals as the fat is heaped up in the cell. This again is directly opposed to the finding of Waldvogel in phosphorus poisoning.

An examination of the nitrogen and phosphorus pentoxide percentage of the fatty extracts indicates that somewhat wide variations are present, the nitrogen varying from 0.3 to 3.1 and the phosphorus pentoxide from 1.1 to 7.3. It is evident, however, that the lower percentages are in those experiments in which the greatest amount of fat is present (Experiments 18, 43, 27 and 21). With these exceptions the percentage figures are quite regular. Only one explanation can be made of this difference. That is, that as fatty compounds make their appearance in the cell, those substances predominate which do not contain nitrogen or phosphorus, namely the neutral fats, fatty acids and cholesterol.¹⁷

Of particular interest is the ratio of phosphorus to nitrogen discussed above. It is seen from an examination of the figures in the table that this ratio remains remarkably constant regardless of the absolute variation in the figures. The ratio, with the exception of Experiment 5 which is high, varies only from 1:2.4 to 4.9. If we also exclude Experiments 21 and 27 the variation is still further reduced to 1:3.5 to 4.9, which is well within the limits of error or better perhaps within the calculation error as made from the published figures for the nitrogen and phosphorus percentages of such poorly defined substances as protagon and jecorin. This high ratio indicates that the nitrogen- and phosphorus-containing fats present in the extracts are of the protagon and jecorin type and remain so irrespective of the amount of fat appearing in the cell.

It must be admitted that some slight evidence does exist to indicate that perhaps under certain conditions these bodies may undergo autolysis. In the two experiments, for example 21 and 27 with a low ratio (1:2.4 to 3.0), considerable autolysis must have been going on during the extensive necrosis and the low ratio as a concomitant factor would point to the appearance in the extract of bodies such as the lecithins, which as previously stated possess a ratio of 1:1. In these experiments roughly one third of the nitrogen- and phosphorus-containing fats has been replaced by lecithin, according to this reasoning. We are somewhat sceptical, however, of the validity of this line of argument since it does not

¹⁷ We possess confirmatory evidence upon this point in the results which have been obtained with the saponification equivalent. These will be published in a later paper.

agree with Waldvogel's observations; nor is the low ratio present in the two experiments (28 and 29) where one would expect it to be most markedly diminished since the necrosis was most pronounced. In these two instances, however, the ratio is normal. As the low ratio occurs in only two out of the eight experiments in which necrosis was present, we are more inclined to believe that some other factor is the true cause for the change.

SUMMARY.

1. Changes which occur in the fat content of the liver of dogs receiving hæmotoxic serum bear no relation to the degree of necrosis produced by this serum.

2. An increase in water content of the tissue seldom occurs, but where present is due to the nitrogenous autolysis rather than to the deposition of fat.

3. The appearance of fat in the cell is not associated with a decomposition of the proteid component of the compound fats, but rather to a simple splitting off of the fatty radical. This is shown by the slight variations occurring in the percentage nitrogen of the fat-free substance.

4. The iodine equivalent diminishes as the fat content increases. This would indicate that in the fatty changes which occur, fats other than those containing oleate radicals make their appearance.

5. The ratio of phosphorus to nitrogen in the alcohol-chloroform extract remains practically constant in all degrees of necrosis. Hence the substances of the protagon and jecorin type hold the same relation to the lecithins during the autolysis as they do normally.

6. In a general way it may be said that the results obtained in the microchemical staining of the fats with Scharlach R agree with those found by chemical extraction methods.

BIOLOGICAL RELATIONSHIPS OF DIPLOCOCCUS
INTRACELLULARIS AND GONOCOCCUS

By MARTHA WOLLSTEIN, M.D.

BIOLOGICAL RELATIONSHIPS OF DIPLOCOCCUS INTRACELLULARIS AND GONOCOCCUS.¹

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Recent studies by Brickner and Cristéanu² having shown a marked similarity between *Diplococcus intracellularis* and gonococcus, as instanced by their agglutinin and precipitin reactions, as well as their effect on inoculated animals, it seemed interesting to follow the same lines of work and carry them further by testing for possible specificity of the immune bodies developed in the sera of animals immunized to these two organisms. Some of the experiments made by Dr. Flexner³ on the biology of the diplococcus were repeated with the gonococcus in order to detect possible differences.

BIOLOGY OF THE GONOCOCCUS.

Like the intracellularis, the gonococcus is a coffee-bean shaped diplococcus occurring more frequently within leucocytes than outside them. Comparison of smears from the pus of a recent case of gonorrheal vaginitis and from the cerebro-spinal fluid of an early case of cerebro-spinal meningitis (both in infants) shows a marked similarity, in that both present many polymorphonuclear leucocytes containing from one to ten or more pairs of Gram-negative diplococci, and some pairs of cocci lying extra-cellular. There is, however, a decided difference in the size of the two varieties of organisms, the intracellularis being much the larger of the two. This relatively larger size is also seen in smears made from the peritoneal exudate in guinea-pigs killed by inoculations of diplococcus and gonococcus respectively. In agar cultures, twenty-four hours old, on the other hand, the gonococcus is larger, possibly because its growth is so much less profuse.

¹ Received for publication July 2, 1907.

² Brickner and Cristéanu, *Compt. rend. de la Soc. de Biologie*, 1906, lx, 846; 942; 988; 1070.

³ Flexner, *Jour. of Exper. Med.*, 1907, ix, 105.

Gonococcus grows best upon glucose-serum-agar, prepared by adding about one third its volume of human pleuritic or ascitic fluid to the melted agar. The reaction of the medium proved unimportant unless very alkaline to phenolphthalein, a faintly acid, neutral, or faintly alkaline medium giving about equal growths.

The cultures of gonococcus studied were isolated from cases of vaginitis occurring in young infants at the Babies' Hospital. Smears from the discharge in such cases show the gonococcus to be the only organism present in almost every instance, so that glucose-serum-agar plates made from the pus obtained by passing a platinum loop into the vaginal canal gave, in the majority of instances, an abundant and almost pure growth of gonococcus colonies.

Glucose-agar and dog's serum-agar proved useless as a medium for the cultivation of the first generation of gonococci, but subsequent plants from human serum-agar to these media gave a faint growth consisting of separate colonies surviving from two to five days, and bearing transplantation for from two to six generations only. Sheep's serum-agar proved an excellent culture medium, providing that fully one third volume of serum was added. The sheep's serum-glucose-agar ordinarily used for the growth of *Diplococcus intracellularis* was useless, because it contained too little serum. Thalmann's⁴ agar gave excellent growths of all the strains of gonococci, but the cultures survived only from seven to twelve days. Picker⁵ found that not all strains of gonococcus grew on Thalmann's agar, but some survived twenty-one days, and some even as long as six months, if the tubes did not become dry. The advantage of Thalmann's agar would seem to lie in its reaction, obtained by neutralizing two thirds of the quantity of agar to phenolphthalein and then adding the other, acid, third. Vannod⁶ found that plain agar made slightly alkaline to litmus paper is a very suitable medium for the gonococcus, while if the agar is alkaline to phenolphthalein the organism will not grow upon it. I did not repeat Vannod's experiments.

On human serum-glucose-agar slants gonococci remained viable from twenty-six to thirty-eight days when capped with rubber and

⁴ Thalmann, *Cent. f. Bakt.*, 1900, xxvii, 828; 1902, xxxi, 678.

⁵ Picker, *Wien. klin. Woch.*, 1906, xix, 1282.

⁶ Vannod, *Cent. f. Bakt.*, 1906, xl, 162; 1907, xlv, 10.

kept at 37° C., or as long as the tubes remained moist. The corresponding uncapped cultures in the thermostat usually survived from sixteen to twenty-one days. The addition of a drop of a suspension of calcium carbonate did not prolong the viability of the gonococcus beyond the period of survival on moist serum-glucose-agar, while the danger of making the medium too alkaline with the carbonate is a drawback to its employment.

Gonococcus did not grow in sheep's serum-water containing sugars, but in human serum-water litmus medium to which dextrose or maltose had been added gonococci caused slight reddening without coagulation. Lactose, saccharose, mannite and dextrine were unaffected. In their ability to ferment sugars, gonococcus and intracellularis acted alike. Dunn and Gordon⁷ found that the gonococcus did not affect maltose, thus differentiating the two varieties of cocci. The ten strains I isolated from infants all fermented maltose.

Some of the experiments in viability and autolysis made by Dr. Flexner⁸ with diplococcus were repeated with gonococcus, suspensions being made in salt solution and also in Ringer's fluid, a duplicate series kept in the ice chest and in the thermostat at 37° C. The suspensions were of four different strengths: the original turbid suspension, and the same diluted twice, five and ten times. The results were almost parallel with those obtained by Dr. Flexner with the *Diplococcus intracellularis*, more cocci remaining viable in the concentrated salt solution suspensions kept at 7° C., and more in the weaker suspensions at 37° C. While in Ringer's fluid the larger number of cocci survived in the concentrated suspension at 37° C., and in the weaker suspension at 7° C. Cover slips were made daily from these tubes, and the cocci found to be less disintegrated in the lower than in the higher salt solution suspensions kept in the thermostat, while in the tubes kept in the ice chest there was remarkably less disintegration, many cocci staining well on the sixth day, although growth had ceased on the third from both salt and Ringer's solution suspensions. Growth took place after six days in sub-cultures made from the salt solution suspensions kept at 37°

⁷ Dunn and Gordon, *Brit. Med. Jour.*, 1905, ii, 421.

⁸ Flexner, *loc. cit.*

C., and after seven days from the Ringer's solution suspensions. Cover slips showed rather less daily disintegration in the Ringer's solution than in the salt solution. Among the surviving cocci the larger, more resistant pairs, taking on a deep safranin stain, described by Dr. Flexner, were distinctly seen in the cover slips. As in the case of the intracellularis, cold is more injurious than warmth to the gonococcus. But unlike the intracellularis, Ringer's fluid did not prolong the viability of the gonococcus beyond or even up to the period of survival in ascitic-glucose-agar. Even when kept in the ice chest (at 37° C.) growth was obtained in sub-cultures on the seventh day from the serum-agar tubes, while such tubes kept in the thermostat gave excellent sub-cultures on the twenty-seventh to thirty-ninth days. Cover slips showed many deeply staining cocci at that late day, but most of the cocci had been disintegrated.

Two different strains of the diplococcus grew in sub-cultures made on the thirty-fifth day after inoculation on moist sheep's serum-agar, the tubes having been kept constantly at 37° C. A parallel set of tubes kept in the ice chest gave growth for seven days only and no well-staining cocci could be detected in cover slips made on the eighth day. Thus while gonococcus survives longer on solid media kept at 37° C. than does the *Diplococcus intracellularis*, the viability of both organisms on such media at 7° C. is about the same.

As in the case of the diplococcus, the autolytic ferment of the gonococcus is destroyed by exposure to a temperature of 65° C. for thirty minutes. Suspensions in water, salt solution and Ringer's fluid gave the same results in this respect.

PATHOGENESIS OF THE GONOCOCCUS.

Cultures of gonococcus isolated from cases of vaginitis at the Babies' Hospital were inoculated into white mice and young guinea-pigs. The first or second generation was used to inoculate the surface of a pint Blake bottle of serum-glucose-agar. After twenty-four hours at 37° C. the growth was suspended in four cubic centimeters of 0.9 per cent. salt solution and one half injected into the peritoneal cavity of each of two guinea-pigs weighing between 170 and 200 grams. In this way nine different strains were injected in

the second or third generation. All the pigs died within twenty to twenty-four hours. White mice succumbed to smaller doses of recent cultures, one serum-agar slant being sufficient as a rule to cause death over night, half a tube failing to do this. As the mice reacted very irregularly to the inoculations, they were not used extensively.

Cultures lose their virulence readily. It was found that where a second or third generation had caused death in a guinea-pig (170 to 200 grams) in twenty hours, when given doses of half the surface growth of a pint Blake bottle, the sixteenth or eighteenth generation proved not to produce a fatal result when the entire growth in the bottle was injected.

It has been shown by Bail⁹ that sub-lethal doses of bacteria may become lethal under the influence of fluids containing aggressins, so-called, which remove the natural protective powers of the organism. While Bail and Weil¹⁰ maintain that aggressins are formed and found chiefly, though not exclusively, in the body fluids, and first at the point of inoculation where the bacteria are proliferating most rapidly, Wassermann and Citron¹¹ were able to achieve apparently the same results with bacterial extracts made by shaking cultures in normal rabbit's serum or distilled water; and they insist that aggressins are not newly formed in the animal organism, but are merely a dissolved bacterial substance, which is itself toxic. Bail¹² holds the opinion that the natural aggressins described by him, and the artificial ones obtained by Wassermann and Citron are not identical; but into this discussion I shall not enter.

Only artificial gonococcus and diplococcus aggressins, so-called, were used in my work, the extracts being prepared by suspending the twenty-four hours old growth on the surface of a Blake bottle in five cubic centimeters of salt solution, adding a few drops of toluol and leaving the cocci to autolyze over night at 37° C., after which the resulting fluids were preserved in the refrigerator. Just

⁹ Bail, *Arch. f. Hyg.*, 1905, lii, 272.

¹⁰ Weil, *Cent. f. Bakt.*, 1906, xli, 121. Bail and Weil, *Cent. f. Bakt.*, 1906, xlii, 51.

¹¹ Wassermann and Citron, *Deut. med. Woch.*, 1905, xxxi, 1101. Citron, *Cent. f. Bakt.*, 1906, xli, 230.

¹² Bail and Weil, *Cent. f. Bakt.*, 1906, xlii, 51.

before inoculating, the extract was centrifuged until clear or nearly so, and the toluol removed by evaporation in the thermostat (37° C.). Intracellularis extracts were made with ten cubic centimeters of salt solution because the growths were so much more profuse than those of the gonococcus. Whether the addition of this extract to a sub-lethal dose of the coccus would increase its pathogenicity was tested in two ways: First, non-fatal doses of the coccus and its own extract were used; second, the extract of a recent culture (third generation) was given with the cocci of an old culture (forty-ninth generation) and vice versa.

White mice were used for the cross experiments between old and recent strains. It became apparent that the addition of half a cubic centimeter of extract to a non-fatal dose of a twenty-four hour old culture of its own or the other strain of gonococcus caused the death of white mice within twenty-four hours. Specificity of the aggressin is not limited to the homologous strain of gonococcus.

Working with a strain which did not kill a guinea-pig, weighing 170 to 200 grams, in doses of two cubic centimeters, it was found that the addition of a quarter of a cubic centimeter of its extract made the dose a fatal one. Conversely, two cubic centimeters of the extract proving sub-lethal, the addition of a quarter of a cubic centimeter of a surface growth in a Blake bottle suspended in five cubic centimeters of salt solution caused death within ten to twenty hours. But on decreasing the maximum sub-lethal dose of extract or culture in these combinations the animals did not die regularly within twenty-four hours, so that the invariably fatal dose proved to be two cubic centimeters of suspension of the culture plus one quarter of a cubic centimeter of extract, or vice versa.

Working with a diplococcus which was not fatal in doses of half a cubic centimeter (of a ten cubic centimeter salt solution suspension of a twenty-four hour growth on sheep's serum-agar in a pint Blake bottle), the addition of half a cubic centimeter of the extract of the same coccus caused death within eighteen hours, a smaller dose of either extract or culture proving non-fatal. Nothing less than one cubic centimeter of this extract alone killed over night.

The fatal dose of both the gonococcus and the intracellularis combinations having been determined, cross reactions were made. The

suspensions of intracellularis and gonococcus were always made as nearly equal in strength as possible. It was found that a larger dose of gonococcus culture, two cubic centimeters, was required to make half a cubic centimeter of diplococcus extract fatal, while only half a cubic centimeter of diplococcus culture sufficed to make two cubic centimeters of gonococcus extract kill within twenty hours; more was needed than of intracellularis culture to raise the power of the other organism to the fatal point. Thus it becomes evident that the aggressive action of the extracts of gonococcus and diplococcus is more potent for its own than for the other variety of coccus, though the two may act interchangeably in larger doses, and hence are not specific. Dörr¹³ has shown the lack of specificity of many bacterial aggressins (coli, dysentery, cholera, pyocyaneus, staphylococcus), and Paul and Lotti¹⁴ found a certain quantitative but not qualitative specificity among them. Bail¹⁵ and Salus,¹⁶ on the other hand, maintain that the natural aggressins are strictly specific.

To prove whether inoculation with living gonococcus cultures or with their extracts protected against intracellularis cultures and extracts, and vice versa, pigs which survived the above experiments were later given a lethal (or larger) dose of the other organism. The diplococcus extract alone, and also non-fatal combinations of extracts and culture did not protect against a fatal dose of a recent diplococcus culture given five to twenty-eight days later. Sublethal doses of intracellularis culture (0.05 to 0.2 cubic centimeter) protected against a fatal dose given seven to nine days later, while less (0.025 cubic centimeter) did not protect. The gonococcus culture alone, and the culture plus the extract, enabled pigs to survive a fatal dose of diplococci injected thirteen to thirty days later. Combinations of gonococcus cultures and intracellularis extract protected against a lethal intracellularis dose administered two to five days later. Not only is specificity lacking here, but the gonococcus alone or in combination with its extract seems to be a more powerful

¹³ Dörr, *Wein. klin. Woch.*, 1906, xix, 759; 1038; 1081.

¹⁴ Paul and Lotti, *Cent. f. Bakt.*, 1907, xliii, 718; 809.

¹⁵ Bail, *loc. cit.*

¹⁶ Salus, *Wien klin. Woch.*, 1906, xix, 870.

protection against fatal doses of living diplococci than the diplococcus itself.¹⁷

The anatomical lesions found in guinea-pigs dying within twenty-four to thirty-six hours after inoculation with living gonococcus cultures are very similar to those described by Dr. Flexner¹⁸ in pigs which succumbed to intracellularis injections. There are marked œdema of the pancreas and surrounding tissues, congestion or hæmorrhage of the adrenals, small hæmorrhages into the mesentery, serous coat of the intestines and the parietal peritoneum, with more or less clear or turbid fluid in the peritoneal cavity, and a layer of pus and fibrin over the liver, spleen and omentum. An increased amount of clear fluid in the pleural cavities is often noted. Cover slips from the peritoneum and omentum show varying numbers of polymorphonuclear leucocytes and of diplococci, within and outside these cells. Multiplication of the cocci is more in evidence after inoculation with both culture and extract than when culture alone is injected, and phagocytosis is much less marked under those conditions. When the extract alone has been administered neither cocci nor leucocytes appear in the cover slips, and cultures remain sterile.

SERUM REACTIONS.

Precipitins.—Four sera were tested for precipitin reactions. Three were from rabbits immunized to the gonococcus, and one from a rabbit inoculated ten times with the intracellularis. The sera were tested from twenty to twenty-seven hours after bleeding and the cocci were prepared in four different ways: the sodium hydrate (0.15 per cent.) macerations recommended by Brickner and Cristéanu;¹⁹ salt solution macerations prepared in the same way; salt solution toluol extract described by Flexner;¹⁸ and the filtrate of Thalmann's broth used by Torrey.²⁰ Cultures of diplo-

¹⁷ The reactions just described call for a special study in order to establish their significance. The failure of the *Diplococcus intracellularis* to induce resistance or immunity, may be due to a slower final recovery period than in the case of the gonococcus. The reaction noted of the gonococcus versus the diplococcus may, possibly be of the nature of the non-specific reactions of resistance produced by such an indifferent body as bouillon which also endure for a brief period of time.

¹⁸ Flexner, *loc. cit.*

¹⁹ Brickner and Cristéanu, *loc. cit.*

²⁰ Torrey, *Jour. of Med. Research*, 1907, xi, 329.

coccus, gonococcus and *Micrococcus catarrhalis* were used, and normal fresh rabbit's serum as control. Only one serum (from a rabbit receiving nine inoculations of living gonococci) gave any precipitin reaction, and that only in dilutions of one to ten and one to twenty for both gonococcus and diplococcus.* It gave no reactions with *M. catarrhalis*. The normal serum and salt solution controls were always negative. Two other anti-gonococcus sera and one anti-diplococcus serum gave negative precipitin reactions, yet all these sera showed the presence of immune body as demonstrated by the deviation of complement (*vide infra*). Muir and Martin²¹ have shown that the formation of precipitate is not a necessary accompaniment of the phenomena of complement deviation in antisera. Brickner and Cristéanu¹⁹ found the precipitin reactions with gonococcus and intracellularis in anti-gonococcus serum to be identical. Torrey²² finds an appreciable difference between the two.

Agglutinins were not high in amount in any serum obtained by immunizing rabbits with increasing doses of gonococcus and diplococcus over periods of eight to ten weeks. Six sera were examined: (a) agglutinated both gonococcus and intracellularis in dilutions of one to ten before inoculation, and in one to fifty after seven injections of living cocci; (b) agglutinated one to twenty before inoculation and both gonococcus and diplococcus in dilutions of one to four hundred after ten injections of living cocci; (c) agglutinated one to ten before inoculation; after ten doses of gonococcus extract, gonococci were agglutinated in dilutions of one to one hundred, diplococci only in one to fifty dilutions; (d) agglutinated in one to ten before treatment, ten doses of living gonococci and extract injected, after which both gonococcus and diplococcus were agglutinated in dilutions of one to fifty; (e) did not agglutinate either coccus before treatment; eleven inoculations of living diplococci developed agglutinins for both gonococcus and diplococcus in dilutions of one to four hundred, one to six hundred was negative; (f) agglutinated in dilutions of one to twenty before inoculation, and after nine doses of living gonococci the serum gave positive agglutination with gonococci in dilutions of one to one hundred, and with intracellularis, one to twenty.

²¹ Muir and Martin, *Jour. of Hyg.*, 1906, vi, 265.

²² Torrey, *loc. cit.*

Bruck's²³ statement that inoculations with living cultures produce a serum rich in agglutinins but poor in amboceptors seems to be borne out in only two of the four sera so produced in my experiments. But the sera showing the highest agglutination reactions were both obtained with living cultures. One of them, however, showed the highest amboceptor content of any serum studied. On the other hand, the sera produced by means of inoculations with extracts with or without living cocci showed very low agglutinations.

The anti-diplococcus serum kindly supplied by Dr. Jobling was from a horse which is being inoculated with cultures and extracts of *Diplococcus intracellularis*. It agglutinated with the intracellularis in dilutions of one to one hundred, and gonococcus, one to fifty.

Brickner and Cristéanu²⁴ obtained exactly the same amount of agglutination with diplococcus and gonococcus with the serum of a horse inoculated with gonococci. Vannod,²⁵ on the other hand, found a marked difference in the degree to which these two organisms agglutinated in their respective specific sera, and believed that while group agglutinins exist, a large number of specific ones also exist. Torrey's²⁶ results led him to the opinion that the agglutinins for gonococcus and intracellularis are common in very low dilutions only. As no one of my sera agglutinated its homologous coccus (grown on Thalmann's agar) in dilutions higher than one to four hundred, the contrast with Torrey's positive reactions in dilutions of one to two thousand to one to seven hundred thousand after nine or ten inoculations is very marked.

Deviation of Complement.—Müller and Oppenheim²⁷ were the first to demonstrate specific anti-bodies in the serum of a male (adult) case of gonorrheal arthritis by means of the complement deviation test, normal human serum being used as controls.

Bruck showed the presence of specific immune bodies for gono-

²³ Bruck, *Deut. med. Woch.*, 1906, xxxii, 1368.

²⁴ Brickner and Cristéanu, *loc. cit.*

²⁵ Vannod, *Deut. med. Woch.*, 1906, xxxii, 1984.

²⁶ Torrey, *loc. cit.*

²⁷ Müller and Oppenheim, *Wien. klin. Woch.*, 1906, xix, 894.

cocci in the serum of three adult cases of gonorrhœal disease (two females and one male). Agglutinins and precipitins were lacking in all of them. He also called attention to the existence of such amboceptors in the serum of inoculated rabbits.

Vannod²⁸ used gonococcus nucleo-proteid for the immunization of rabbits, and obtained a serum which agglutinated gonococcus in dilutions of one to three hundred and contained sufficient specific immune bodies to inhibit hæmolysis by deflecting complement when added in proportion of 0.01 cubic centimeter to the serum. Another serum, agglutinating at one to four hundred, prevented hæmolysis when present in a dilution of 0.025 and 0.001 cubic centimeter. He thinks that agglutinins and specific anti-body develop side by side, not independently, as Bruck²⁹ has stated.

Four sera were tested for specific immune bodies by means of Bordet and Gengou's³⁰ method of complement deviation in the presence of antigen, using a hæmolytic system as the indicator. The free receptors in the extract (antigen) having been bound to the complement by the amboceptor (specific) present in the immune serum to be tested, the addition (after one hour) of an inactivated lytic serum and its corresponding corpuscles caused no hæmolysis. In the present tests, washed hen's corpuscles in five per cent. solution and rabbit's serum made lytic to hen's corpuscles were used. Fresh guinea-pig serum was employed as complement, in amounts varying from one twentieth to one fortieth of a cubic centimeter. Normal horse serum and normal rabbit serum were used as controls, as three of the immune sera were from rabbits and one from a horse. The immune sera and the extracts were tested with corpuscles alone and with hæmolytic system alone, with and without complement, before being combined with either.

I. Serum 494, from a rabbit inoculated with gonococcus extract in salt solution. This serum inhibited hæmolysis by deviating complement in dilutions of one to fifty, with one tenth of a cubic centimeter of extract (Table I). The results were identical when diplococcus extract was used. Having found the minimum amount of amboceptor necessary to bind the complement to the receptors in

²⁸ Vannod, *loc. cit.*

²⁹ Bruck, *loc. cit.*

³⁰ Bordet and Gengou, *Ann. de l'Inst. Pasteur*, 1901, xv, 289.

the extract and thus prevent hæmolysis, this amount was doubled and the antigen titrated against it. Then it developed that 0.05 cubic centimeter of immune serum deviated complement in dilutions of antigen from 0.1 to 0.002 of a cubic centimeter (Table II); and, going further by titrating the serum against the smaller dose of extract, 0.005 cubic centimeter sufficed to inhibit hæmolysis in the presence of 0.05 to 0.0005 cubic centimeter of immune serum (Table III). Below this dilution hæmolysis was complete. As the results were identical with gonococcus and diplococcus extracts with anti-gonococcus serum, it follows that the amboceptor in that serum was as readily bound to the pre-receptors in the intracellular extract as to those in the gonococcus extract, and strict specificity is, therefore, lacking. I am indebted to Dr. Jobling for the suggestion to titrate immune serum and antigen successively against double the smallest binding dose of either. In this way smaller amounts of immune body were demonstrable. It was hoped, also, to bring out specific differences between the effect of the two extracts upon the serum, but none such were obtained.

Normal rabbit serum with similar dilutions of antigen showed no inhibition of hæmolysis. The following tables embody the above results.

TABLE I.

Immune Serum No. 494.	Complement Guinea-pig	Antigen		Anti-hen Rabbit's Serum.	Hen's Corpuscles.	Results.
		Gonococcus.	D. Intracellularis			
0.1	0.05	0.1	0	0.01	0.05	—
0.05	"	0.1	0	"	"	—
0.02	"	0.1	0	"	"	—
0.01	"	0.1	0	"	"	±
0.005	"	0.1	0	"	"	+
0.1	"	0	0.1	"	"	—
0.05	"	0	0.1	"	"	—
0.02	"	0	0.1	"	"	—
0.01	"	0	0.1	"	"	±
0.005	"	0	0.1	"	"	+

The sign + means complete hæmolysis; — no hæmolysis; and ± incomplete hæmolysis.

The following controls, nine in number, were made with every series of tests. I give them here instead of repeating them in each

Controls.

No.	Immune Serum.	Guinea-pig Complement.	Antigen.	Anti-hen Serum.	Hen's Corpuscles	Results.
1	0	0.05	0.1	0.01	0.05	++
2	0.5	0	0.1	0.01	0.05	—
3	0.5	0.05	0	0.01	0.05	++
4	0	0	0	0.01	0.05	—
5	0	0.05	0	0.01	0.05	++
6	0	0	0.1	0	0.05	—
7	0.5	0	0	0	0.05	—
8	0	0.05	0	0	0.05	—
9	0	0	0	0	0.05	—

TABLE II.

Immune Serum No. 494.	Guinea-pig Complement.	Antigen.		Anti-hen Rabbit's Serum.	Hen's Corpuscles.	Results.
		Gonococcus.	D. Intracellularis.			
0.05	0.025	0.1	0	0.01	0.05	--
"	"	0.05	0	"	"	—
"	"	0.02	0	"	"	—
"	"	0.01	0	"	"	—
"	"	0.005	0	"	"	—
"	"	0.002	0	"	"	—
"	"	0.001	0	"	"	+
"	"	0	0.1	"	"	—
"	"	0	0.05	"	"	—
"	"	0	0.02	"	"	—
"	"	0	0.01	"	"	—
"	"	0	0.005	"	"	—
"	"	0	0.002	"	"	—
"	"	0	0.001	"	"	+

TABLE III.

Amboceptor Immune Serum No. 494.	Guinea-Pig Complement.	Antigen.		Anti-hen Serum.	Hen's Corpuscles.	Results.
		Gonococcus Extract	D Intracellularis Extract			
0.05	0.025	0.005	0	0.01	0.05	—
0.02	"	"	0	"	"	—
0.01	"	"	0	"	"	—
0.005	"	"	0	"	"	—
0.002	"	"	0	"	"	—
0.001	"	"	0	"	"	—
0.0005	"	"	0	"	"	—
0.0002	"	"	0	"	"	+
0.05	"	"	0.005	"	"	—
0.02	"	"	"	"	"	—
0.01	"	"	"	"	"	—
0.005	"	"	"	"	"	—
0.002	"	"	"	"	"	—
0.001	"	"	"	"	"	—
0.005	"	"	"	"	"	—
0.002	"	"	"	"	"	+

table. It is hardly necessary to say that only when these controls were correct were the results of the experiments admitted. The quantity of immune serum and antigen varied in each series of controls according to the test to be made. The amount of complement, corpuscles and antigen serum were the same throughout.

2. Serum 495. A second anti-gonococcus rabbit's serum, obtained after ten injections of gonococcus extract and cultures, was found to be anti-hæmolytic without antigen whenever guinea-pig complement was employed. As the serum had been inactivated by heating to 54° C. for thirty minutes, it was thought that anti-hæmolytic substances might have developed under the influence of heat. But on using rabbit complement, hæmolysis was complete, even with 0.2 cubic centimeter of the serum.³¹ One tenth cubic centimeter of antigen inhibited hæmolysis in the presence of 0.001 cubic centimeter of immune serum (Table IV). In titrating anti-

TABLE IV.

Amboceptor Immune Serum No. 495	Rabbit Serum Complement.	Antigen.		Anti-hen Rabbit's Serum.	Hen's Corpuscles.	Results.
		Gonococcus Extract.	D. Intracellu- laris Extract.			
0.1	0.05	0.1		0.01	0.05	—
0.05	"	0.1		"	"	—
0.02	"	0.1		"	"	—
0.01	"	0.1		"	"	—
0.005	"	0.1		"	"	—
0.002	"	0.1		"	"	—
0.001	"	0.1		"	"	—
0.1	"	0	0.1	"	"	—
0.05	"	0	0.1	"	"	—
0.02	"	0	0.1	"	"	—
0.01	"	0	0.1	"	"	—
0.005	"	0	0.1	"	"	—
0.002	"	0	0.1	"	"	—
0.001	"	0	0.1	"	"	—
1.0005	"	0	0.1	"	"	—

³¹ Presumably the guinea-pig serum contained free receptors to which the amboceptor in the immune rabbit serum anchored the complement, thus preventing hæmolysis when the hæmolytic serum was added. On saturating the immune serum with guinea-pig corpuscles over night in the ice chest, and centrifuging the next day, the anti-hæmolytic power of the serum was found to have been lost. The same result was brought about by using rabbit instead of guinea-pig serum as complement.

gen against 0.002 cubic centimeter of immune serum, some differences in the results with the two extracts were noted, the gonococcus being the stronger of the two (Table V).

TABLE V.

Amboceptor Immune Serum No. 495.	Rabbit Serum Complement.	Antigen.		Anti-hen Rabbit's Serum.	Hen's Corpuscles.	Results.
		Gonococcus Extract.	D. Intercellu- laris Extract.			
0.002	0.05	0.1	0	0.05	0.05	—
"	"	0.05	0	"	"	—
"	"	0.02	0	"	"	—
"	"	0.01	0	"	"	—
"	"	0.005	0	"	"	—
"	"	0.002	0	"	"	—
"	"	0.001	0	"	"	—
"	"	0.0005	0	"	"	—
"	"	0.0002	0	"	"	±
"	"	0	0.1	"	"	—
"	"	0	0.05	"	"	—
"	"	0	0.02	"	"	—
"	"	0	0.01	"	"	—
"	"	0	0.005	"	"	—
"	"	0	0.002	"	"	—
"	"	0	0.001	"	"	+
"	"	0	0.0005	"	"	+

3. Serum 4. A third anti-gonococcus serum was obtained from a rabbit immunized with living cocci. Amboceptor for gonococcus was present in a dilution of one to ten thousand in the presence of one tenth cubic centimeter of extract, and one to two thousand for intracellularis. Table VI gives the results of this and further titrations of the extract.

It is evident that the highest amboceptor content for gonococcus was present in the anti-serum obtained by immunizing a rabbit with living gonococcus cultures, agglutinins being also higher than in the other two sera from rabbits immunized with extracts, and with extracts plus cocci. In these high dilutions a distinct difference between the amount of gonococcus and diplococcus amboceptor was apparent, although the two ran parallel until a dilution of one to two thousand was reached.

The anti-diplococcus horse serum obtained from Dr. Jobling inhibited hæmolysis completely in dilutions of one to five hundred with one tenth cubic centimeter of diplococcus antigen, and incompletely with that amount of gonococcus antigen. Doubling the

TABLE VI.

Amboceptor Immune Serum No. 4.	Guinea-Pig Serum Complement.	Antigen.		Anti-hen Serum.	Hen's Corpuscles.	Results.
		Gonococcus Extract.	D. Intracellu- laris Extract.			
0.1	0.05	0.1	0	0.01	0.05	—
0.05	"	"	0	"	"	—
0.02	"	"	0	"	"	—
0.01	"	"	0	"	"	—
0.005	"	"	0	"	"	—
0.002	"	"	0	"	"	—
0.001	"	"	0	"	"	—
0.0005	"	"	0	"	"	—
0.0002	"	"	0	"	"	—
0.0001	"	"	0	"	"	—
0.00005	"	"	0	"	"	±
0.1	"	0	0.1	"	"	—
0.05	"	0	0.1	"	"	—
0.02	"	0	0.1	"	"	—
0.01	"	0	0.1	"	"	—
0.005	"	0	0.1	"	"	—
0.002	"	0	0.1	"	"	—
0.001	"	0	0.1	"	"	—
0.0005	"	0	0.1	"	"	±
0.0002	"	0	0.1	"	"	+
0.0001	"	0	0.1	"	"	—
0.001	"	0.1	0	"	"	—
0.001	"	0.05	0	"	"	—
0.001	"	0.02	0	"	"	—
0.001	"	0.01	0	"	"	—
0.001	"	0.005	0	"	"	—
0.001	"	0.002	0	"	"	—
0.001	"	0.001	0	"	"	—
0.001	"	0.0005	0	"	"	—
0.001	"	0.0002	0	"	"	—
0.001	"	0.0001	0	"	"	—
0.001	"	0	0.1	"	"	±
0.001	"	0	0.05	"	"	—
0.001	"	0	0.02	"	"	—
0.001	"	0	0.01	"	"	—
0.001	"	0	0.005	"	"	—
0.001	"	0	0.002	"	"	—
0.001	"	0	0.001	"	"	—
0.001	"	0	0.0005	"	"	—
0.001	"	0	0.0002	"	"	±
0.001	"	0	0.0001	"	"	+

inhibitory amount and titrating the two extracts, the results proved to be identical, deviation being complete with 0.02 cubic centimeter of antigen, incomplete with 0.005 cubic centimeter, and absent with a smaller quantity. On testing the inhibitory amount of antigen with diminishing amounts of amboceptor, it was found that 0.01 cubic centimeter was required to prevent hæmolysis with intracellularis, and 0.02 cubic centimeter for gonococcus. Neither the

amboceptor content nor the agglutinins of this serum were high, and the differences for the two varieties of cocci were very small.

Extracts of *Micrococcus catarrhalis*, of *Streptococcus pyogenes*, of a Gram negative diplococcus from a dog, of the typhoid bacillus and of two varieties of dysentery bacilli (Shiga and Flexner types) did not inhibit hæmolysis in combination with this diplococcus immune serum. Normal horse serum controls with gonococcus, diplococcus, streptococcus, *Micrococcus catarrhalis*, and the typhoid and dysentery bacilli were all completely hæmolyzed.

An anti-typhoid serum obtained from Dr. Park at the Board of Health deviated complement in a combination of one tenth cubic centimeter with one two hundredth cubic centimeter of typhoid antigen, but it had no effect upon hæmolysis when combined with intracellularis, gonococcus, streptococcus or *Micrococcus catarrhalis*.

On several occasions extracts of gonococcus and of diplococcus became useless after about two weeks, because they inhibited hæmolysis in doses of one tenth cubic centimeter or less. This was found to be due to the presence of protectin,³² which fact was demonstrated by shaking the extract with ether for two hours, decanting, evaporating over a water bath and taking up the residue in salt solution. Two tenths and one tenth of a cubic centimeter of this suspension protected blood corpuscles from solution in the presence of their inactivated lytic serum and fresh complement. I am indebted to Dr. Noguchi for this demonstration.

CONCLUSIONS.

The most marked differences, exclusive of pathogenic effects in man, between gonococcus and *Diplococcus intracellularis* are cultural ones, and consist chiefly in abundance of growth and choice of medium.

Relatively larger doses of gonococci than of diplococci are required to kill young guinea-pigs, but the lesions are very similar in the two cases, and both organisms lose pathogenic power rapidly when cultivated artificially.

Agglutinins, aggressins, protective power, and the amboceptors

³² Noguchi, *Jour. of Exper. Med.*, 1906, viii, 726.

developed in the serum of immunized animals seem to be largely common to both diplococcus and gonococcus.

Neither other Gram negative cocci nor *Streptococcus pyogenes* have any receptors in common with intracellularis and gonococcus.

My thanks are due to Dr. Flexner for suggesting and supervising the work, and to Dr. Jobling for many courtesies.

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**IS DEATH IN HIGH INTESTINAL OBSTRUCTION
DUE TO THE ABSORPTION OF BILE? ***

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Fellow of the Rockefeller Institute.

It is well known that acute intestinal obstruction occurring in the duodenum or oral portion of the jejunum is much more rapidly fatal than a similar obstruction occurring aboral to this portion of the intestine.

A rather careful study of the literature of the subject has failed to show that any definite cause has been discovered for this well known fact, everything to date being suppositional in character. So recent and so high an authority as M. Wilms, whose extensive monogram on ileus has just appeared, in discussing high intestinal obstruction says: "The time in which duodeno jejunal obstruction causes death has only in the rarest instances been prolonged. Death usually subvenes with extreme rapidity. All patients probably succumb to toxic absorption resulting from the decomposition of intestinal and stomach contents."

Starvation and lack of absorption of water, which has been thought by some to be a factor in producing the syndrome of duodeno jejunal obstruction, are hardly to be considered, particularly when one reflects that absorption of water takes place almost entirely from the colon and can therefore not be materially influenced by the position of the obstruction in the small gut. For, as Mayo says, we drink with our great gut and eat with our small. Wilms makes no mention of the possible relationship of the biliary and pancreatic fluids as a cause of death in duodeno jejunal obstruction.

Neither are these the only suggestions which have been offered as an explanation of the phenomena under discussion.

* A Research conducted under a Fellowship granted by the Rockefeller Institute.

Boszeky and Genersich offer two theories to explain the symptoms in intestinal obstruction, viz., reflex and auto intoxicational. They believe that bacteria and their toxins pass through the intestinal wall; that the slow pulse and other phenomena are caused by the interference with the vagi and splanchnics, which causes congestion of the abdominal organs, thus giving rise to anemia elsewhere, noticeably in the brain. The anuria which is characteristic of this condition is due in the opinion of these two authors to exhaustion of vaso dilators of kidneys and the drop in temperature to exhaustion of the heat centre, all due to cerebral anemia. "But," they continue, "in cases of obstruction by enteroliths, these symptoms cannot be explained on the ground of nervous phenomena. Here it must be chiefly an accumulation of feces and ptomaines and toxins due to *putrefaction of intestinal contents*."

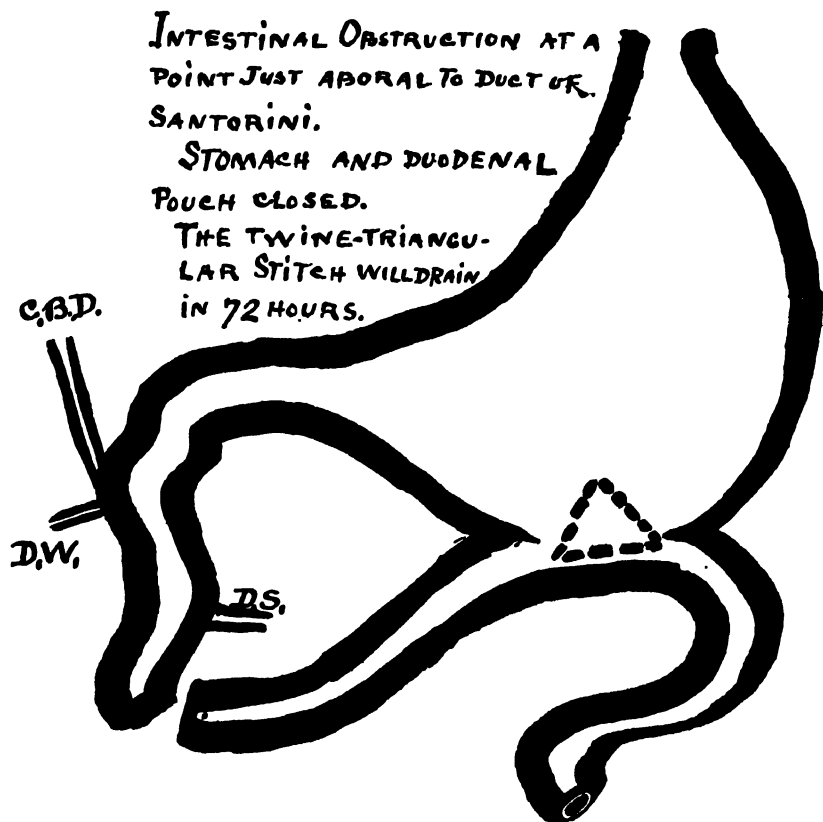
Here again in an exhaustive German monograph attention is called to the importance of putrefaction of the intestinal contents. Observations which we have made in the Laboratory show conclusively that putrefaction has nothing whatsoever to do with the cause of death in intestinal obstruction, at least when the seat of obstruction is within 35 centimeters of the pylorus.

Accidentally in the beginning, and, more recently, volitionally, we have studied this obscure but exceedingly important problem. It will be necessary in order to make our results clear, to give a short history of the manner in which the subject has been approached.

In a series of experiments conducted in the Surgical Research Laboratory of Columbia University and which were carried on to find some practical method of performing a gastroenterostomy by the so-called closed method, it was noticed that under certain constant conditions the animals operated on invariably suffered a similar train of symptoms which were always followed by a pseudo tetanic form of death. The pathological picture marked by fibrillary muscular twitchings, weakness and later rigidity is not unlike that presented after a parathyroidectomy. The conditions which

united to cause this precise form of death are shown in the accompanying outline (Fig. 1). They consisted in the performance of a gastroenterostomy by a closed method and the severance and closure of the short duodenal loop. The actual technic of effecting the establishment of the stoma is not of immediate concern here. It was made by what has been called

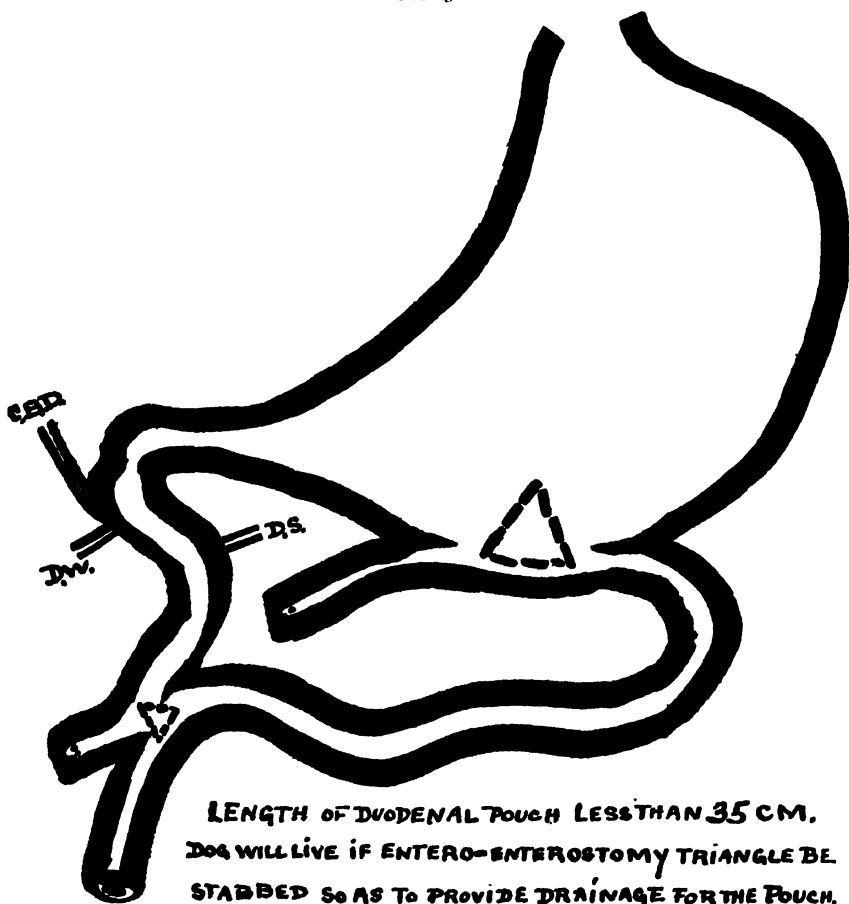
FIG. 1.



in previous communications the *twine triangular stitch*. It is necessary, however, to emphasize the fact that this triangular stitch, which is a simple substitute for the McGraw elastic ligature, has been introduced in all the series as a control. It is of great use for experimental purposes when it is desired to close the stomach for a constant number of hours and subse-

quently to have drainage re-established. The twine cuts a punched out opening at a period of from 70 to 100 hours. This varies in different subjects, but in long series a general average will be maintained. This is the first point to make clear.

FIG. 3.



In the second place, it was found that the length of the loop from the pylorus to the invagination was the essential factor in determining whether or not the animal would live. It was shown after many tests that if the distance from the infolding to the pylorus was less than 35 centimeters, it would die before



the twine triangular stitch gave drainage (70 to 100 hours). If the loop were longer, the animal would live.

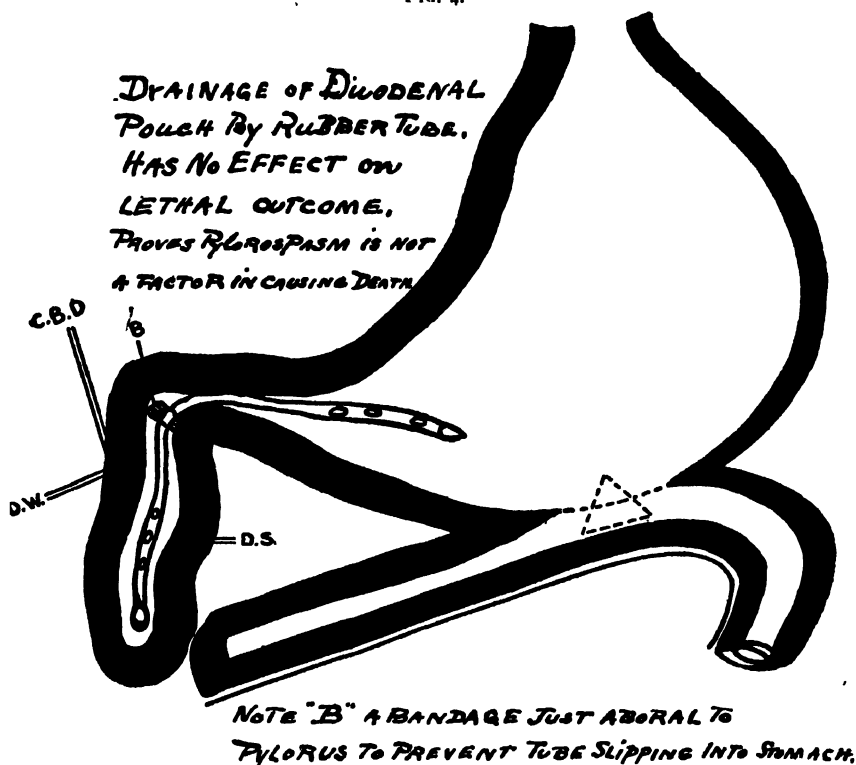
The third point is this: It was suggested by Dr. Flexner that this pseudo-tetanic death might be ascribed to decomposition of food materials in the stomach and duodenal loop. A number of experiments, however, upon fasting animals demonstrated that the presence or absence of food material in the stomach had no effect whatsoever in modifying the influence already alluded to of length of loop. Animals with stomachs well filled and those with alimentary canals thoroughly emptied showed no variation whatsoever in character or rapidity of death. The lethal line in either case was always approximately 35 centimeters from the pylorus.

There was a fourth point of importance. The question arose: Was the disturbance of innervation at the pylorus, responsible for the death of the animal? A series of six dogs were operated on in the following manner. The pylorus was either sectioned or resected together with a small amount of stomach, and the ends infolded, great care being taken not to injure the bile duct. Twine triangular ligature was then inserted to create a gastroenterostomy. Without exception these animals lived until after the stoma had been established. This led to a most important conclusion. It showed that if the section and blockage of the duodenum were made oral to the bile duct, so that drainage were maintained for hepatic and pancreatic secretions, the animal would tolerate the absolute closure of the stomach without any ill effect until this viscus were drained by the establishment of the stoma 70 hours later. It is of great import to notice this, for it shows that, the nervous element owing to shock produced by section of the gut in the neighborhood of the pylorus, which has long been recognized to be a serious factor from a vital standpoint, in all operations in this neighborhood has absolutely nothing to do with this form of death which we have described as pseudo-tetanic. It suggests that the biliary or pancreatic secretions, or both, stand in some very definite relation to the lethal results observed. Pseudo-tetanic death occurring so constantly after section aboral to

these ducts ~~and so rarely~~ when the section was made oral to them, is indicative that some profound interruption of normal chemical processes in the intestine produces the fatal result rather than any nervous disturbance or shock.

It should be emphasized that the animals will live with a blocked loop less than 35 centimeters in length if the stoma be made an open one. Drainage of the stomach into the intestines

FIG. 4.



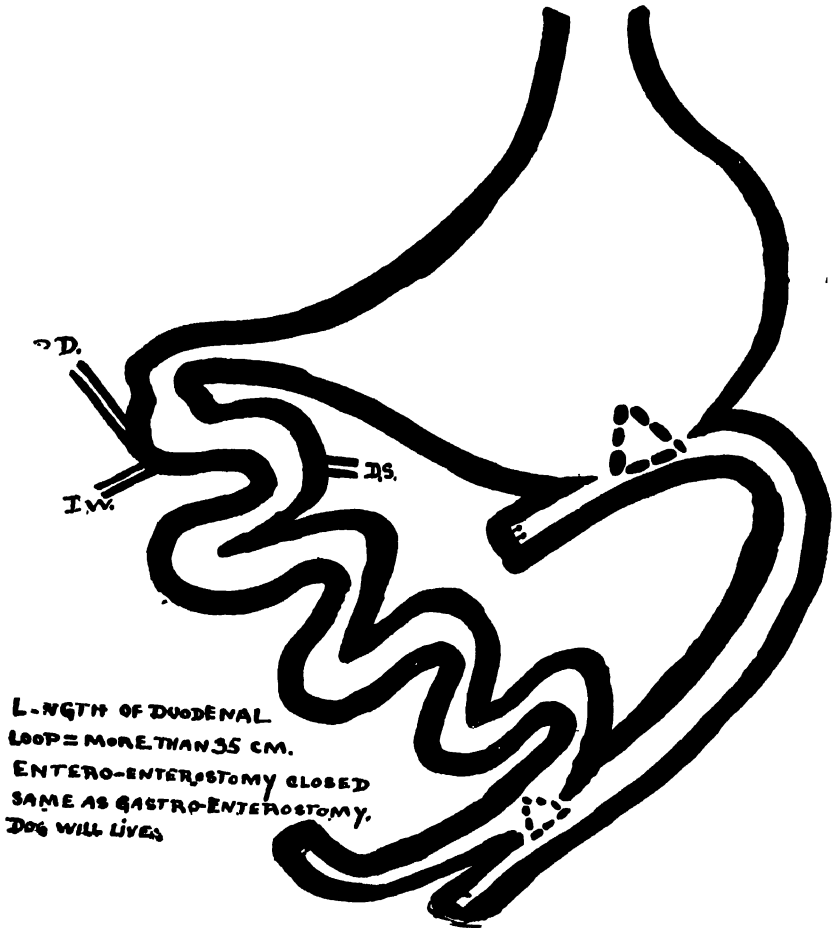
suffices to save the animal's life, no matter where the duodenum may be blocked.

It was further determined that the effect of entero-anastomosis between the jejunum just distal to the gastroenterostomy and the closed duodenal loop just aboral to the entrance of the ducts gave a similar result. Dogs operated on in this manner live. In other words, drainage of the loop is as

effectual in preventing the lethal results ~~as is~~ drainage of the stomach.

Moreover the fatal outcome is not the result of a closure of the loop produced by pylorospasm. The figure (4) shows how the proof of this was obtained. Rubber tubes placed so

FIG 5. .

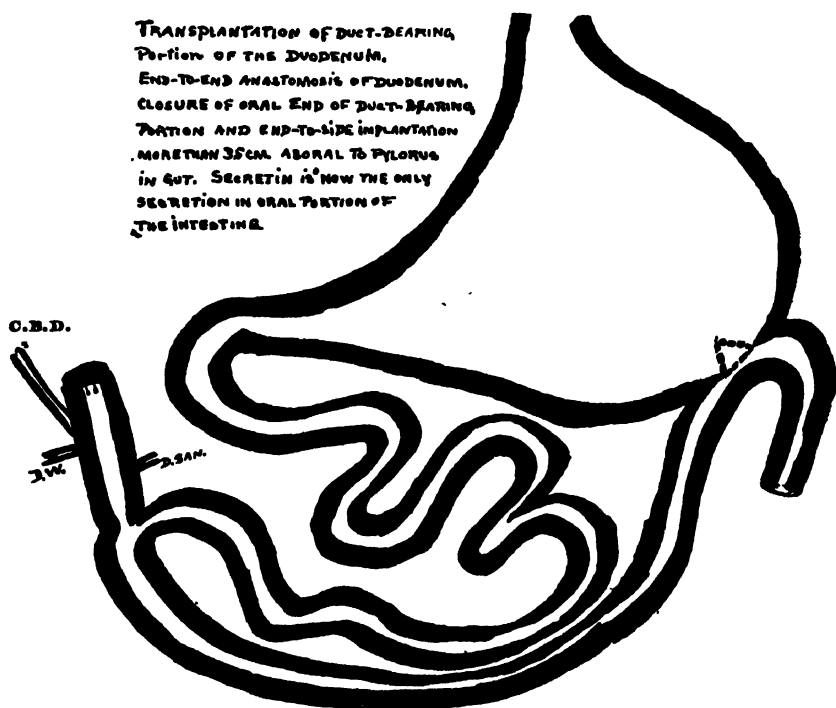


as to connect the loop with the stomach failed to be instrumental in preventing death. Drainage of the loop into the stomach is therefore shown to be without effect in preventing death.

Having eliminated nervous shock, as the source of lethal

impulse, as well as poisoning from food decomposition, obviously it became evident that the cause of death might lie in the production of poisonous substances produced by a mixture of the biliary and pancreatic secretions. It seemed possible, moreover, that this hypothesis might give us the real explanation of the fact that 35 centimeters or more of intestine in the closed duct-bearing portion sufficed to avert fatal results. For we may conceive that in this length of intestine such a quantity

FIG. 6.



of diluting material may constantly be furnished as would be adequate to render harmless the mixture alluded to. This supposition seems to be corroborated by the recent discovery of Flexner that the biliary salts, unless in colloidal suspension, act as violent poisons. He states (Jour. Exper. Med., 1896, p. 174) that the conclusion may be drawn that the suspension of bile in a bland mixture of high colloidal strength protects

the pancreas from the immediate and acutely injurious action of the biliary salts."

Further, on page 167, he continues: "Bile contains two sets of constituents of highly different chemical composition crystalline principals and colloids. The biliary salts are known to act injuriously upon the cells while no direct cellucidal property is known either for the biliary coloring matter or the mucin."

None the less convincing as to the toxicity of the bile are the studies of Meltzer and Salant. They state: (Jour. Exper. Med., 1906, 8, p. 159) "We have positive and direct proof that normal bile from many rabbits possesses an exciting element capable of producing chronic convulsions in frogs." Page 165, "that contrary to the prevailing opinion, bile contains a tetanic element or an agent which causes increase of excitability of the nervous system. That stagnant bile as in the gall bladder, etc., invariably produces coma and paralysis, that the depressive and exciting elements of the bile are mutual antagonists; that the depressive element when present in a highly effective dose is by far the stronger of the two, while on the other hand the tetanic element becomes effective apparently in a dose far below that which constitutes the minimum for the depressive element. That bile salts seem to contain the tetanic element in an extremely less amount than the whole bile."

Moreover and also in support of the supposition that a true auto intoxication from biliary or pancreatic products is responsible for death in high intestinal obstruction, we quote from *Opie* (Diseases of the Pancreas). He showed by a long series of experiments that the introduction of bile into the pancreas caused either death of the animal within twenty-four hours or widespread destruction of the gland depending upon the amount of bile injected.

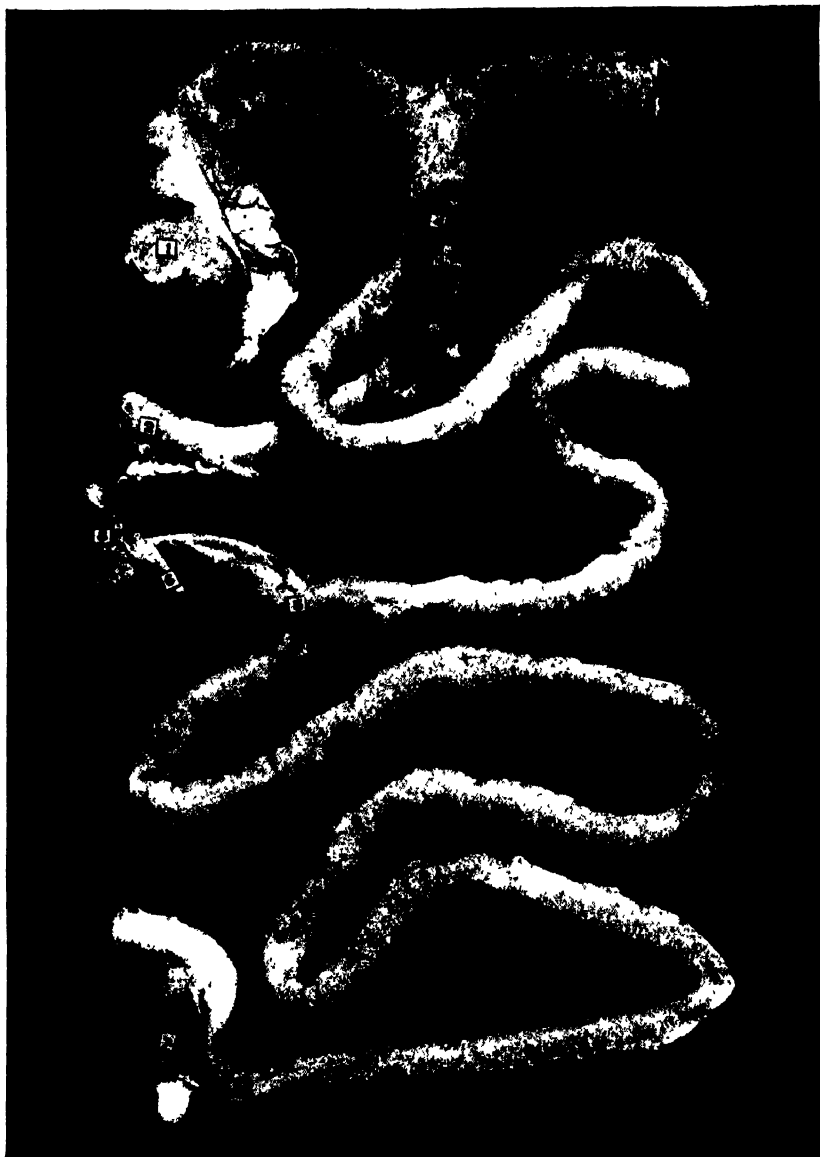
As a means of further study of this question, Dr. Blake suggested the possibility of transplanting the duct-bearing portion of the duodenum into the intestine at varying distances from the stomach. This is accomplished by means of section between the bile duct and the pylorus of the first portion of the

duodenum and a second section of the duodenum aboral to the entrance of the duct of Wirsung. The segment of gut thus removed is carefully sponged out and wrapped in hot cloths. The ends of the pylorus and duodenum are then united by end to end suture. More than 35 centimeters aboral to the pylorus an end to side implantation of the segment is done, the oral end beside the bile duct having been carefully invaginated. This is a difficult operation, but it can be brought to successful issue. Dogs emaciate quite rapidly after it has been done, so that the most recent operations practised have included a section of the intestine just aboral to the implantation of the duct bearing segment, and a gastroenterostomy by means of the triangular stitch between the stomach and the gut aboral to the section. This, it will be noticed by consulting the figure, produces the same results from an operative standpoint as is shown in Fig. 1, except that the loop of intestine from the bile duct to the pylorus is more than 35 centimeters in one case, while in Fig. 1, it is not more than 10 or 12 centimeters. These experiments are not as yet concluded.

A simpler method of testing the effect of the presence of the secretions would naturally be to tie the pancreatic ducts and the bile duct. This is easier from a technical standpoint, but of course it does not produce conditions in any way resembling the normal as does the transplantation technic already referred to. Nevertheless in a number of experiments, both the ducts of Santorini and of Wirsung have been tied as well as the bile duct. This series is not yet far enough advanced to enable us to make a report upon it. Some of the dogs have died and on autopsy the pancreas has been found to be exceedingly hard, while the omentum has been dotted with unmistakable areas of fat necrosis. This gross evidence of pancreatic lesion has not been observed in our cases of pseudo-tetanic death occurring after duodenal section and infolding just aboral to the ducts. It suggests therefore that the form of death in the one case may have been different from that in the other.

In another series of experiments, the bile duct alone has

FIG. 7.



Photograph of specimen obtained from a dog in which acute duodenal obstruction was established. Cholecystenterostomy and twine triangular gastro-enterostomy and section of common duct were done at same time. Pancreatic ducts intact. Dog appears to have lived because of transplantation of the bile from duodenum into ilium. 1, Duodenal pouch. 2, Infolded aboral end. 3, Pancreas. 4, Site of stoma. 5, Cholecystenterostomy. 6, Cut end of common duct. 7, Cecum. 8, Remnants of liver. Mounted by Dr. Brown.

been tied with two ligatures and divided between them. Here again the series is as yet too short to enable us to make any positive statements, but several dogs in which the bile ducts were cut, have lived with a short duodenal pouch and twine triangular stoma.

A method for transplanting the point of bile entrance beyond the 35 cm. line and one less difficult as well as less apt to be accompanied by adventitious pathological conditions such as fat necrosis is a simple chole cystenterostomy. This has the added advantage of separating the bile and pancreatic secretion. It is not easy to do this operation in two steps because of the formation of troublesome adhesions and done at one sitting, prolonged as it must be by gut section and infolding and by the triangular enterostomy, there is danger of death from shock or later by peritonitis from leakage. We have done two of these one-stage operations without a death by shock, but followed by fatal peritonitis on the fourth day. The third dog lived three weeks, and was killed to obtain the specimen shown in Fig. 7. It is immaterial that the animals died of peritonitis—the point of interest is that they survived beyond the time which would have been possible had the bile been passing down its normal channel.

MacCallum of Johns Hopkins who, with associates, has given us a most elaborate and exact study on ileus inclines to the belief that death is due to the absorption of bacterial toxins, which enter through the impaired gut wall. Clinically Mayo and others have confirmed this assumption by observing a lowered mortality to follow a wide resection of the dilated gut. Nevertheless it seems noteworthy, first, that total exclusions of gut segments may be made which, left in the abdominal cavity gradually grow to great size (see paper of Blake & Brown in this issue) without producing any signs of toxemia; second, that this work has been done in the relatively germ-free portion of the gut; third, that obstruction is tolerated during the cutting out of the control if placed oral to the bile duct while it always is fatal before the control cuts out, if situated aboral to it.

In conclusion it may be said that so far as our experiments go, there seems reasonable ground to believe that they demonstrate that death in duodeno-jejunal obstruction may be due to the absorption of toxic elements in the bile which are normally rendered harmless by dilution and colloidal suspension in the secretions of the small intestine. That as the length of small intestine from the bile duct to the site of obstruction decreases, so the diluting secretions decrease and the toxicity rises proportionately. That if further experimentation definitely proves that bile is directly the cause of death in certain forms of intestinal obstruction, it may be possible to lower the operative mortality. For a knowledge of the pathology of a condition is the first step toward the establishment of a successful mode of treatment.

THE ELIMINATION OF RADIUM FROM NORMAL AND NEPHRECTOMIZED ANIMALS.¹

BY WILLIAM SALANT² AND GUSTAVE M. MEYER.³

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INTRODUCTION.

DURING the past fifty years numerous studies of the fate of foreign substances after their introduction into the body have shown conclusively that the kidney is not the sole organ of elimination for such substances. Metallic and other elements, as well as complex organic compounds, if given by mouth or injected into the circulation, pass from the organism not only by way of the kidneys, but may be excreted by the digestive organs, by the skin, in the lachrymal or other secretions, or by various additional routes.

Thus the large number of investigations of the fate of iron in man and other animals, no matter in what form or by what channel it may have been administered, have shown that the large intestine is practically the only channel of elimination for this element. The same was shown for manganese and certain other heavy metals whose elimination has been studied. Somewhat similar data have been obtained for the alkali earth metals. Thus, calcium, strontium, and magnesium, although eliminated in the urine after their injection, are found in much larger quantities in the contents of the intestines. Various other elements, which are excreted chiefly by the kidneys,

¹ The fifth paper in a series of researches with radium inaugurated by Dr. WILLIAM J. GIES in this laboratory several years ago. See (1) BERG and WELKER: *Journal of biological chemistry*, 1906, i, p. 371; (2) BURTON-QUITZ and MEYER: *Journal of experimental medicine*, 1906, viii, p. 245; (3) MEYER: *Journal of biological chemistry*, 1906, ii, p. 461; (4) HUSSAKOF: *Medical record*, 1907, lxxii, p. 89. See also a recent report of an unfinished research in the series, by GAGER: *Science*, 1907, xxv, p. 462.

² Fellow of the Rockefeller Institute for Medical Research.

³ A preliminary report appeared in *Science*, 1907, xxv, p. 459.

are, to some extent, also eliminated by the intestinal epithelium. The work of Good¹ has shown that lithium chloride injected hypodermically into cats and dogs, although largely excreted in the urine, is also found in the saliva as well as in the stomach and in the intestinal contents. Hanford² found that cesium is mainly eliminated by the kidneys, but it was also detected, after subcutaneous as well as after intravenous injections, in the saliva, in the contents of the stomach, in the contents of the intestine throughout its entire length, and also in the bile. Likewise, rubidium, which leaves the body mainly in the urine, was found by Mendel and Closson³ in small quantities in the feces. Such examples might be multiplied.

That the gastro-intestinal wall also serves as an organ for the elimination of complex organic bodies has been demonstrated in the cases of various substances, such as morphin, quinin, atropin, curarin, snake venom, and other toxic compounds of biological origin. These particular substances seem, according to several investigators, to be eliminated by the gastric epithelium as well as by the kidney.

That the bile is an important channel of elimination of various substances introduced into the body has been shown by several workers. After the intravenous injection of albuminate of zinc into cats, dogs, and rabbits, Michaelis⁴ found zinc in the bile of these animals. Mosler⁵ detected iodine in the bile of dogs eight hours after the administration of potassium iodide. He obtained the same results when he gave potassium iodide to a patient with a biliary fistula. Wichert⁶ studied the elimination in the bile of a large number of elements when introduced into the body. Potassium iodide or potassium bromide was given by mouth to cats and dogs. After a short time the bile of these animals showed the presence of iodine or bromine respectively. Similar results were obtained with antimony, nickel, bismuth, lead, silver, and arsenic. In this connection may be mentioned the experiments of Mead and Gies⁷ in this laboratory with tellurium. According to these investigators this element is eliminated not only by the kidneys, but also by the liver, and even by the

¹ GOOD: *American journal of the medical sciences*, 1903, cxxv, p. 273.

² HANFORD: *This journal*, 1903, ix, p. 235.

³ MENDEL and CLOSSON: *This journal*, 1906, xvi, p. 152.

⁴ MICHAELIS: *Archiv für physiologische Heilkunde*, 1851, x, p. 127.

⁵ MOSLER: *VIRCHOW'S Archiv*, 1858, xiii, p. 29.

⁶ WICHERT: *Dissertation*, Dorpat, 1860, xiii, p. 29.

⁷ MEAD and GIES: *This journal*, 1901, v, p. 104. Also Gies and collaborators: *Biochemical Researches*, 1903, i, Reprint No. 21.

lungs. Organic bodies of complex chemical constitution may likewise be eliminated in the bile. According to Brauer,¹ methylene blue, when given by mouth, is excreted into the bile. Meyer² has lately carried out in this laboratory a large number of experiments with various anilin dyes. All of them were eliminated in the bile.

It is evident, therefore, from the foregoing very general *résumé* that foreign substances, if introduced into the body, may be ejected through many exits.

The results of the recent work here with barium by Berg and Welker,³ and with radium and anilin dyes by Meyer,⁴ led us all in this laboratory to believe that various conclusions pertaining to the paths of elimination of certain substances, inorganic compounds particularly, have been jumped at too hastily by a number of workers. Thus, Meyer⁵ has lately drawn attention to what appears to be an unwarranted deduction by Mendel and Sicher⁶ on the excretion of barium in the urine, — unwarranted because the experimental data offered by Mendel and Sicher failed to support it. With regard to the elimination of foreign substances through the intestinal mucosa, it seems that in many cases the experimental evidence did not support the announced positive conclusion to that effect. In some of the instances alluded to, such a conclusion appears to have hinged on the assumption that the presence of a given substance in the intestinal contents indicates the excretion of that substance through the intestinal wall, — a deduction that is assuredly fallacious in view of the fact that metallic and other elements have been detected in such gastro-intestinal streams as saliva, bile, and pancreatic juice. Hence the excretory function of the intestine can be accurately determined in a particular case only after the passage of all such secretions into the intestines has been prevented. Meyer is at present engaged in such an investigation of the excretion of barium, and the companion study described in this paper is a further outcome of our feeling here that these questions need more thorough experimental study than many of them have received.

Although evidence is not wanting that the intestines are capable

¹ BRAUER: *Zeitschrift für physiologische Chemie*, 1903, xl, p. 182.

² MEYER: *Journal of the American Chemical Society*, 1907, xxix, p. 892.

³ BERG and WELKER: *Journal of biological chemistry*, 1906, i, p. 371.

⁴ MEYER: *Loc. cit.* Also, *Journal of biological chemistry*, 1906, ii, p. 461.

⁵ MEYER: *Journal of biological chemistry*, 1906, ii, p. 474.

⁶ MENDEL and SICHER: *This journal*, 1906, xvi, p. 147.

of eliminating various foreign substances introduced into the body, the need of additional data obtained in the way suggested above is very desirable. Moreover, since therapeutic measures, based on the assumption that the gastro-intestinal mucosa eliminates foreign substances that are of no use to the organism, are frequently resorted to by clinicians, a study of elimination through the stomach as well as through the bile was also desirable. On account of the facility with which very minute amounts may be sharply detected in the tissues and fluids of the body, Professor Gies suggested the use of radium for the study of our problem.

EXPERIMENTAL.

Methods.— Our experiments were carried out on dogs and rabbits under ether narcosis. A permanent biliary fistula was established in one dog (Experiment 2). In all the other animals bile was obtained from temporary fistulæ. To prevent the passage of saliva into the stomach and intestines during an experiment, a ligature was placed at the cardiac end of the stomach. Ligatures were likewise placed at the pylorus, and below the opening of the duct of Wirsung, also at the junction of the large and small intestines in the dog. In each rabbit the upper and lower ends of the cecum were closed by ligatures.

In each experiment radium bromide (1000 activity), in aqueous solution, was injected *subcutaneously*. Bile and urine were usually collected. The contents of the different sections of the gastro-intestinal canal were carefully removed at the end of an experiment, and examined for radium by the quadrant electrometer. A detailed description of methods for the detection of radium in animal tissues and fluids has already been described by Meyer.¹ The same methods were employed in this investigation.

Experiment 1. Dog; weight, about 10 kilos. 10 mg. of radium bromide were injected. About two hours later the dog was killed. The bile collected from the gall bladder was radioactive.

Experiment 2. Dog; weight, about 10 kilos. A permanent and complete gall bladder fistula was established on June 11, at 4 P. M. The dog made a very good recovery from the operation. On June 18th the stitches were removed, and the fistula was apparently well healed. During the collection of bile the dog was placed in the holder described in the preceding paper.

¹ Meyer: *Journal of biological chemistry*, 1906, ii, p. 462.

June 25th, 2.30 P. M. 30 mg. of radium bromide were injected into the left leg. The bile obtained several hours later was radioactive. Urine and feces collected separately were likewise radioactive.

June 27th, 4 P. M. The dog was killed by chloroform narcosis. The stomach was found empty. Small pieces of the gastric wall were removed for examination. The contents of the intestines as well as small pieces of the wall of the intestines were also separately tested for radium. The results of the examination showed that neither the stomach nor the intestines were radioactive. The contents of the intestines, however, were radioactive.

Experiment 3. Female dog; weight, 8 kilos. 10.30 P. M.: ether anesthesia. The stomach was ligated at the cardiac and pyloric ends. The small intestine was ligated at some distance below the opening of the common duct. The large intestine was ligated at the junction of cecum and appendix. The neck of the gall bladder was clamped and a cannula placed in the common bile duct. 11.15 A. M.: 20 mg. of radium bromide were injected into the right leg. 11.15-1.15 P. M.: 10 c.c. bile were collected. 1.15-5 P. M.: 7 c.c. bile were collected. Urine was removed from the bladder at 5 P. M. The dog was killed at 6 P. M.

Both samples of bile as well as the urine were radioactive. The stomach and contents separately examined failed to show the presence of radium. The contents of the small intestine included between the pylorus and the opening of the duct of Wirsung were active; the wall of this part of the intestine was inactive. The record of the test for radioactivity of the remaining portion of the small intestine was unfortunately lost. The contents of this portion proved to be radioactive. The large intestine as well as the contents separately examined were both inactive. The blood was also examined and proved to be radioactive.

These experiments show, therefore, that in the dog the liver as well as the kidney is able to eliminate radium. The behavior of the gastro-intestinal tract is especially worthy of remark in this connection. Neither the stomach nor the large intestine excreted radium. Elimination of this element seemed to take place, however, all along the small intestine.

The advisability of studying elimination in the rabbit now suggested itself, since it has been shown by Noel Paton and Bergmann that herbivora behave differently from carnivora in respect to elimination. Thus Paton's¹ experiments with phosphoric acid have shown that none of it is excreted in the urine of goats, whereas

¹ PATON, N., DUNLOP and AITCHISON: *Journal of physiology*, 1900, xxv, p. 212.

Bergmann¹ made the interesting observation that in dogs phosphoric acid is almost entirely excreted by the kidney, while in sheep elimination takes place through the intestines.

Experiment 4. Female rabbit; weight, 2.2 kilos. 11 A.M.: ether narcosis.

A cannula was placed in the common bile duct. Ligatures were attached as follows: Above the cardiac and immediately below the pyloric ends of the stomach, below the duct of Wirsung, above and below the cecum. At 12 M., 10 mg. of radium bromide were injected into the right leg. Bile was collected during the following periods:

I	12-1	P. M.	10 C.C.
II	1-4	"	15 "
III	4-8.30	"	14 "
IV	8.30-9	A. M.	15 "
Urine obtained at	1	P. M.	10 "
	4	"	10 "

At 9 A.M. the next morning the rabbit was found dead. 15 c.c. of bile were secreted after 8.30 P.M. the previous day. The stomach and intestines were then removed from the body of the animal and carefully washed free from all adherent blood. The contents of the various portions of the gastro-intestinal canal were washed into clean casseroles, and in each case thoroughly mixed before portions of them were placed in trays in preparation for the tests for radium. The results of the examination were the following:

Bile, sample I,	Radioactive
" II,	"
" III,	"
" IV,	"
Urine, sample 1,	Not "
" II,	Radioactive
Stomach,	Radioactive
Contents of " ,	Not radioactive

The part of the small intestine included between ligatures placed at the pylorus and beyond the duct of Wirsung was not radioactive. The contents of this part were radioactive. The rest of the small intestine as well as the contents separately examined were radioactive. Neither cecum nor contents were radioactive. The large intestine was inactive, while the contents were radioactive.

¹ BERGMANN: Archiv für experimentelle Pathologie und Pharmakologie, 1901, xlvii, p. 77.

Experiment 5. Male rabbit (gray); weight, 2 kilos. Ether narcosis. Cannula in the common bile duct. Ligatures were placed as follows: At the cardiac and pyloric ends of the stomach, and above and below the cecum. The bladder was emptied. 15 mg. radium bromide were then injected into the left leg at 1 P. M. Bile was collected as follows:

1-2 P. M. 8 C.C.

2-5.15 " 11 "

At 9 A. M. next day the rabbit was found dead. 12 c.c. of bile were secreted after 5.15 P. M. of the previous day. The stomach and intestines were removed from the body of the animal and treated as described above.

The bile as well as the urine collected at the end of the first hour after the injection of radium bromide was only slightly radioactive. Radioactivity of the bile and urine secreted during the next three hours was, however, very marked. Another sample of each of these secretions obtained before the death of the animal was only slightly radioactive. The stomach as well as its contents, separately examined, failed to show any radioactivity. Examination of the small intestine for radium likewise proved negative, but its contents were markedly radioactive. Neither cecum nor large intestine showed radioactivity, while the contents in both cases were only slightly radioactive. The blood which was obtained from the heart was also tested. The results were negative.

Analysis of the data obtained in our experiments with rabbits indicates that in these animals elimination of radium invariably takes place through the liver, the kidneys, and the small intestines, after its introduction into the circulation. Moreover it is worthy of remark in this connection, that the liver and the kidneys are apparently equally efficient as organs for the excretion of radium, since, as was shown in experiment 5, the elimination of this element began in both organs at about the same time and probably continued for equal periods. Quite different was the behavior of the stomach, the cecum, and the large intestine with regard to the excretion of radium. In neither of the two rabbits experimented on was there any indication that radium had been eliminated through the stomach. Although the wall of the stomach was radioactive, the contents of the stomach failed to show the presence of this element. The cecum presented some variation in this respect. Radioactivity of the contents in one rabbit was slight, but was altogether absent in the other,—an indication that this part of the intestine, at least in normal rabbits, is variable in its activity as an organ for the elimination of radium. The large intestine of the

rabbit seems likewise to vary somewhat in this regard in different individuals. Thus, while radioactivity of the contents of this part of the intestines was very marked in one rabbit, it was slight in the other, suggesting that this part of the gut does not eliminate radium with equal facility in all rabbits. If the results of our observations on dogs and rabbits are now compared, the following interesting resemblances and differences in the manner of elimination of radium in these animals may be seen.

By referring to the table on page 376 we see that the liver as well as the kidney participated in the elimination of radium in the dogs and rabbits. The gastro-intestinal canal, however, exhibited differences in this connection. While the stomach in each of the animals failed to excrete radium, elimination invariably took place through the wall of the small intestine. The large intestine, on the other hand, acted dissimilarly in the dog and rabbits. Thus, in the dog the portion of the intestine which, it will be remembered, is the organ for elimination of iron, manganese, and certain other elements, did not excrete radium. In the rabbits, on the contrary, elimination through the large intestine did take place, although the rate of elimination seemed to vary in different individuals. If deductions based on the evidence presented on the foregoing pages are warranted, we may conclude that the elimination of radium takes place chiefly through the liver, the kidneys, and the small intestine, and, to a lesser extent also, through the large intestine in some of the herbivora.

At this stage of the investigation it occurred to us that a study of the elimination of radium from nephrectomized rabbits would be very desirable. Although considerable experimental evidence has accumulated to indicate that even when the function of the kidney is unimpaired, the elimination of foreign materials through the digestive tract and the fluids entering it, may take place, the study of the passage of substances through these channels in disease of the kidney or when the urinary passages have been blocked, has made but little progress. The few available experimental data on this subject suggest, however, that organs other than the kidney may by way of compensation eliminate foreign substances from the body under such conditions. Claude Bernard¹ in his experiments with potassium ferrocyanide has shown that this substance which is normally excreted through the kidney, may pass into the saliva if the renal arteries or the ureter

¹ BERNARD, CLAUDE (quoted by ACHARD et LOPEZ) : Séances et memoires de la Société Biologique, March 15, 1902.

have been ligated. In this connection may also be mentioned the fact that in advanced cases of nephritis, urea may be eliminated through the digestive tract, the lungs, the skin, and in the lachrymal and other secretions. It was of interest, therefore, to study whether those parts of the gastro-intestinal canal, such as the stomach and cecum, which fail to excrete radium in normal rabbits, might eliminate this element if the kidneys were removed. Our experiments were carried out on two healthy, full-grown rabbits on which the operation for double nephrectomy was performed under ether narcosis and elimination of radium studied in the way indicated in the previous experiments.

Experiment 6. Male rabbit; weight, 2.24 kilos. Both kidneys were removed by the abdominal route, and a cannula was introduced into the common bile duct immediately afterward. Ligatures were placed at the cardiac end of the stomach, around the duodenum, immediately below the pylorus, at the junction of the cecum and small intestine, and at the junction of cecum and large intestine. 10 mg. of radium bromide were then injected into the left leg. The bile collected during a period of thirty minutes after the injection of radium was not radioactive. The next sample collected during the succeeding hour was active. A third sample, obtained nine hours after the administration of radium bromide, was also active. The rabbit died ten hours after double nephrectomy had been performed. The stomach and the various parts of the intestines were carefully taken from the body of the animal, washed free from adherent blood, the contents removed and put into zinc trays, which were treated as previously described. In each case the organ and its contents were separately examined for radium.

The tests of the various parts of the digestive tract for radium have shown the following results: Stomach as well as contents were slightly radioactive. Both the small intestine and its contents were also radioactive, whereas the cecum as well as its contents failed to show the presence of radium. The large intestine and contents were both radioactive. The results obtained show therefore that elimination through the liver is not accelerated as a result of removal of both kidneys, for the bile obtained during a period of thirty minutes after radium was administered was not yet radioactive. The results obtained with stomach contents, on the other hand, show that there is a tendency to vicarious elimination, for the contents were slightly radioactive. In this regard the intestines behaved the same as those of normal rabbits. The contents of both the large and small intestines showed the presence of radium, whereas the contents of the cecum, as in both normal rabbits, were free from radium.

Experiment 7. Female rabbit; weight, about 2.5 kilos. Double nephrectomy was performed by the abdominal route. Ligatures were placed at the cardiac and pyloric ends of the stomach, at the junction of small intestines and cecum, and at the junction of large intestines and cecum. A cannula was introduced into the common bile duct. At 12.35 P. M. 10 mg. of radium bromide were injected. Bile removed at 1.10 P. M. was not radioactive. Another sample obtained between 1.10 and 2.10 P. M. was radioactive. At 5.30 P. M. the rabbit was bled to death. The bile removed shortly before the death of the animal was still radioactive. The various sections of the gastro-intestinal canal were removed and treated as in the other experiments. Tests for radium showed that neither stomach nor contents were radioactive. The small intestine was likewise inactive, but its contents were radioactive. Examination of the large intestine as well as the contents gave in both instances negative results. In this experiment, therefore, the stomach failed to assume any vicarious function. Moreover, the large intestine, which in all the other rabbits eliminated radium, has in this case behaved in a rather anomalous manner. Contrary to expectations, the excretion of radium was entirely inhibited.

The results of our experiments with nephrectomized rabbits do not answer the question whether the vicarious elimination of radium might be expected to occur in most rabbits. The recent work of Meltzer and Lucas¹ furnishes strong evidence, however, that the gastro-intestinal canal fails to eliminate more readily, after the removal of the kidney, certain substances introduced into the body. Thus, when magnesium sulphate was administered to nephrectomized rabbits, in much smaller doses than those given without special effect to normal rabbits, deep anesthesia was produced. It is probable, therefore, that while vicarious elimination may take place through the walls of the alimentary canal after extirpation, or in diseases, of the kidneys, in the cases of various organic substances produced within the body as a result of metabolism, or when introduced from without, it apparently fails to occur in the cases of certain inorganic substances, which normally leave by other channels. It is quite possible that the liver and small intestine, which normally excrete radium, suffice to meet this need of the body after removal of the kidney.

Finally, we desire to call attention to the study of elimination in the urinary bladder. Although some experimental evidence on absorption from the bladder exists, we could not find any reference in the litera-

¹ MELTZER and LUCAS: Proceedings of the Society for Experimental Biology and Medicine, 1906, iv, p. 10.

ture to an investigation bearing on excretion through this organ. It seemed to us, therefore, that the question ought to be put to an experimental test. Our results indicate that radium was not excreted through the wall of the urinary bladder.

A summary of our results for radioactivity is appended.

TABLE GIVING THE RESULTS FOR RADIOACTIVITY OF VARIOUS PARTS IN EXPERIMENTS 2-7.

Experiment No.	Normal dogs.		Normal rabbits		Double nephrectomized rabbits.	
	2	3	4	5	6	7
Stomach . . .	Inactive	Inactive	Active	Inactive	Slightly active	Inactive
Stomach contents	Inactive	Inactive	Inactive	Slightly active	Inactive
Intestine . . .	Inactive					
Contents of the intestine . .	Active					
Bile	Active	Active	Active	Active	Active	Active
Feces	Active					
Blood	Active	Active	Inactive	Slightly active	
Kidney	Active					
Urine	Active	Active	Active	Active		
Small intestine	Inactive	Active	Inactive	Active	Inactive
Contents small intestine	Active	Active	Active	Active	Active
Large intestine	Inactive	Inactive	Inactive	Active	Inactive
Contents large intestine	Inactive	Active	Slightly Active	Active	Inactive
Cecum	Inactive	Inactive	Inactive	Inactive
Contents of cecum	Inactive	Slightly active	Inactive	Inactive

SUMMARY OF CONCLUSIONS.

In dogs and rabbits the kidney, the liver, and the small intestine eliminate radium. In normal rabbits elimination also takes place through the large intestine. The passage of radium through the wall of the large intestine is probably slower than through the wall of the small intestine. Elimination of radium into the cecum of the rabbit is slight and in some individuals may fail altogether.

In a nephrectomized rabbit the elimination of radium takes place through the small intestine and through the liver. The rate of radium excretion from the livers of rabbits is not affected by removal of the kidneys. In a nephrectomized rabbit elimination of radium through the large intestine is uncertain; in such a rabbit radium is not eliminated through the cecum, but may pass through the stomach wall.

The channels of elimination for radium appear to vary in different species and in individuals of the same species.

After removal of both kidneys from a rabbit there is no compensatory elimination of radium through those parts of the digestive tract which are not concerned in its elimination when the kidneys are intact.

We are indebted to Prof. William J. Gies for numerous suggestions.

**The Altmann's granules in kidney and liver and their relation
to granular and fatty degeneration.**

By **WILLIAM OPHÜLS.**

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Francisco, Cal.]*

In the kidneys of dogs, rabbits and guinea pigs we find the following arrangement of the Altmann's granules: In the connecting, the convoluted tubules and in the descending parts of the loops of Henle, the granules are rather coarse, very definitely rodshaped and arranged in radial rows in the basilar two thirds of the cells, often so closely set end to end that it is difficult to make out the dividing lines between them. In the part of the cells directly adjoining the lumen there are few scattered short rod-shaped granules and none in the "Bürstenbesatz." These details are naturally more plainly shown in the large cells of the convoluted tubules but in a general way the smaller cells in the connecting tubules and in the descending loops of Henle resemble them very closely. Some groups of convoluted tubules have much coarser granules than others. I have not been able to make out whether this is a constant anatomic difference or due to different functional stages. If the granules have any relation to the function of the cells, which seems probable, one would surmise that the connecting tubules cannot purely serve the function of conducting the urine from one place to another, all the more so as in the large ducts in the pyramids which serve this purpose alone, the granules are very scanty and irregularly arranged. In the large light cells of the ascending parts of the Henle's loops the granules are exceedingly small, also slightly rodshaped, extremely numerous and scattered all through the cells in an irregular fashion. This might be used as an argument in favor of a difference in function of this portion of the tubules. In the cells of the liver of these animals the granules vary greatly in size from just visible to quite coarse granules. All of them are rods, some short, others quite long and more or less wavy. The granules are scattered irregularly all over the cells.

In granular degeneration the characteristic macroscopic and microscopic pictures of which can be best produced by intravenous injection of bichromate of potash, the granules enlarge in size, become more or less spherical, lose their normal arrangement and stain very deeply with Altmann's stain contrary to what has been generally assumed after the work of Schilling,¹ who seems to be the only one to have investigated this question. Whether there is an actual multiplication of the granules, it is difficult to decide but on the whole the evidence seems against it. The change is almost exclusively in the convoluted tubules; the connecting tubules and the loops of Henle as a whole are slightly affected if at all. In the liver the change is similar, all cells being equally involved. The albuminous granules in granular degeneration, then, are not new formed granules but largely the enlarged and disarranged normal Altmann granules. I was able to confirm this view in two pronounced cases of parenchymatous degeneration in man.

The relation of the Altmann's granules to fat absorption and fat secretion has already been studied carefully by Altmann himself and his pupils Krehl and Metzner, and they have also touched upon the behavior of the granules in fatty degeneration in phosphorus poisoning. Their conclusion is that fat in all cases appears first in and around the Altmann's granules; they even succeeded in demonstrating remnants of the granules in the center of the initial fat droplets. My observations on the kidneys and liver are confirmatory of these views, although I never succeeded in seeing these remnants of granules in the center of the first fat droplets. It seemed more as if the granules were changed to fat in toto. In fatty degeneration (I use this term for want of a better one) the granules first stain gray with osmic acid and do not take the acid fuchsin stain any more. They may still retain their rod shape. Later they become black and round. The first fat droplets invariably have very nearly the size and in a general way the arrangement of the Altmann's granules. Larger droplets are formed by the fusion of these small ones. I am far, however, from concluding with Altmann that these changes indicate any vital activity in the granules. I should rather imagine that a considerable part of their substance normally must be made up of a

¹ Schilling: *Virch. Arch.*, 1897, cxxxv, p. 410.

combination of fats which does not give the usual reaction of fat and that during fatty degeneration this combination is broken up and the fat liberated.

These observations furnish some explanation why granular and fatty degeneration so frequently occur simultaneously, both being the result of abnormal conditions in the Altmann's granules.

I am greatly indebted to the Rockefeller Institute for financial aid in carrying out these experiments.

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The relation of anatomic structure to function.

By **WILLIAM OPHÜLS**

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It is a well known fact that function is often disturbed without corresponding anatomic lesion. There is always a suspicion, however, that the lack of demonstrable lesion is only apparent and really to be attributed to our crude methods of investigation and our lack of knowledge of the physiologic arrangements. As the Altmann method reveals some very fine details of the protoplasm and as Altmann has shown that during normal function, especially when stimulated by injections of pilocarpin, the appearance and arrangements of the granules, brought out by his methods in the protoplasm, changes quite remarkably, they being in many cases extruded to form part of the secretion, I thought it interesting to see whether these structures would serve as indicators of any primary alteration in the protoplasm of cells during functional disturbances.

The kidney appeared to be the organ best suited for this purpose as by collection of the urine directly after its discharge from the ureters, the exact moment of the occurrence of the disturbance could be ascertained. It is possible to produce albuminuria in dogs within a few hours by intravenous injection of bichromate of potash (about 2-3 c.c. of a 2 per cent. solution). If Altmann's specimens are made from the kidneys at this time no lesions are found. That the poison nevertheless acts upon the epithelial cells and the granules in them is shown by the subsequent development of severe lesion in them.

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In phloridzin glycosuria, likewise, no lesions are demonstrable by this method, although we are fairly certain that the excretion of sugar in this case is due to a lesion in the kidney.

I am inclined to believe that quite a few of the anatomic changes which we now look upon as primary are the result rather than the cause of the functional disturbance, although the disarrangement brought about by them naturally often aggravates the original condition. It is questionable whether the real primary lesion in such cases is of such character as to be ever demonstrable by physical methods.

I am greatly indebted to the Rockefeller Institute for financial aid in carrying out these experiments.

RIGOR MORTIS AND THE INFLUENCE OF CALCIUM AND MAGNESIUM SALTS UPON ITS DEVELOPMENT.*

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THE THEORIES AND SOME OF THE FACTS OF RIGOR MORTIS.

Historical.—Two features of the phenomenon of rigor mortis, the stiffness and the shortening of the skeletal muscles, bear a resemblance to two physiological processes: the clotting of the shed blood and the contraction of living muscles. Upon the basis of this resemblance two divergent theories were brought forward as far back as nearly a century ago. These theories are the ones which at the present day are still competing for supremacy.

The Contraction Theory.—Nysten(1) (1811) thought rigor to be "le dernier effort de la vie contre l'action des forces chimiques." Denuded of its vitalistic garb, the phrase expresses the view that rigor is due to a physiological contraction of the muscles. Although Brücke(2), one of the grave-diggers for the theory of vital force, rejoiced as far back as 1842, at the prospect that the theory of the "dernier effort de la vie" was then buried forever, the theory of contraction was soon resurrected, and even at the present day is occupying a well-supported position in the domain of physiology. Besides Schiff(3), Brown-Sequard(4) and some others, it was especially L. Hermann(5) and his pupils who brought together many facts in favor of the assumption that rigor mortis shows a certain form of muscular contraction.

Coagulation Theory.—As to the coagulation theory it was assumed originally by Orfila(6), Treviranus(7) and others that rigor was due directly to the clotting of the blood and lymph between the

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muscle fibers. This theory was favored also by Johannes Müller(8) for the reason that the contraction of the clot and the separation of serum from it would also explain the final release of the muscles from rigor. This theory, however, had to be given up as soon as it was recognized that rigor also occurs in completely exsanguinated animals. But Brücke offered the same theory in a modified form. Soluble fibrin, said he, enters during life into the muscle fibers and it is the clotting of this intrafibrillar fibrin which is the underlying cause of postmortem rigor. Virchow(9) called attention to the fact that the plasma obtainable from muscle differs chemically from the contents of the blood, and Brücke himself and others failed in the attempt to obtain a plasma from the muscles before they entered into a state of rigor; the chemical theory had therefore for a long period very little support from experimental facts. But in 1859 Kühne(10), who carried out all the necessary manipulations at low temperatures, finally succeeded in obtaining a liquid muscle plasma which gradually clotted spontaneously. The coagulum, which differs from the fibrin of the blood, Kühne termed myosin.

The theory that rigor is due to a sort of coagulation and resembles clotting, or in short the chemical theory of rigor, had now a solid fact for its foundation; the muscles, like the blood, while in a living state, contain a soluble proteid which clots spontaneously some time after the removal from its natural connections. That the rigor is due to the clotting of that proteid within the muscles is, of course, not a fact but only a hypothesis, but a hypothesis which is greatly supported by the following facts: that the spontaneous clotting of the myosin occurs about the same time after death as the muscles, when kept under similar conditions as the plasma, enter into rigor; that further, muscles, from which myosin is pressed out, no longer enter into rigor, and that finally the myosin obtainable from muscles already in rigor is considerably less in quantity than that obtainable from normal muscles. All these facts were brought out by Kühne himself.

Some Facts Favoring the Contraction Theory.—In the half century which passed since the establishment of the chemical theory by Kühne no new facts came to light which essentially strengthened its position, although the knowledge of the chemical nature of

myosin was considerably advanced by many investigators, especially by the extensive studies of Halliburton(11) and von Fürth(12). On the contrary, new questions arose to which the chemical theory is as yet incapable of giving a satisfactory answer. For instance, it is now well established that the clotting of blood plasma occurs under the influence of a well-defined ferment; but no ferment could be discovered, in spite of an eager search for it, which was instrumental in the clotting of muscle plasma(13). Furthermore, is it now shown that the release from rigor is not due to the setting in of putrefaction, as was believed formerly (14 and 15); the release from rigor is as spontaneous an act and as vital a phenomenon, so to say, as its onset. But the once clotted myosin fibrin and myogen fibrin, as the clotted proteid bodies from the muscle plasma are now termed by von Fürth, do not show any reversibility, do not become soluble again. Neither did the vigilant search lead to a detection of a ferment which might be concerned in the release of the muscles from rigor(13). The release reminds one rather of the final relaxation of a muscular contracture. Another fact, difficult to understand on the basis of the chemical theory, is the disappearance of rigor, at least at a certain stage, by bending or kneading the muscles. It was further established that the time of onset, the intensity and duration of rigor differ considerably between white and red muscles (14 and 16). It is known that these muscles differ in the character of their contractility. But it is not known that there is any difference in the fibrin bodies obtained from these muscles. Moreover, the onset and course of rigor depend a great deal upon the antemortem functional state of the muscles; for instance, the antemortem destruction of parts of the central nervous system(17), or cutting of the motor nerves(18), or fatigue of the muscles(19), or their paralysis by curare(20) retard strikingly the onset of rigor; while, after stimulation of motor nerves(21), or after poisoning by strychnin(22), or in deaths due to toxic tetanus(23) the development of rigor is greatly accelerated; all these are facts which indicate an intimate connection of the postmortem rigor with the antemortem muscular contractions.

Various Forms of Rigor.—The numerous studies devoted to the subject brought to light many other valuable and instructive facts,

but it would seem that this knowledge rather increased than diminished the difficulty of interpreting postmortem rigor. In the first place we have to mention other forms of rigor which are to be distinguished from rigor mortis.

1. An extremity of a living animal gets into a state of rigor if its circulation is arrested (Stenon's experiment). This form of rigor, if not too far advanced, is reversible by restoring the circulation.

2. Rigor can further be produced by submersing a muscle in water, or, still better, by injecting water into the arteries of muscles—water rigor(24).

3. By exposing muscles to higher temperatures, they pass rapidly into a state of rigor—heat rigor(25). Quite a large literature grew up on the subject of heat vigor, and there is quite a divergence of opinion as to the nature of the rigor as well as to the degrees of the temperature which cause it in cold-blooded as well as in warm-blooded animals. However, it seems quite certain that rigor occurs at a temperature which is far below the one sufficient to coagulate all the albuminous bodies. Whether there is any relation between these temperatures and the coagulation temperatures of the myosin bodies has not been satisfactorily established. It seems to us that in the literature on heat rigor the conceptions of coagulation and clotting have not always been sharply kept apart,—a confusion against which Kühne had already warned.

4. Rigor can be produced by freezing temperatures—cold rigor (Folin).¹

¹ Folin (*American Journal of Physiology*, 1903, ix, 374) states that Brücke (*Müller's Archiv für Anatomie und Physiologie*, 1842, 176) described rigor produced by cold. But Brücke claims, against Sommer, that rigor persists after thawing and he says nowhere that freezing brings on rigor; on the contrary, he claims that rigor sets in before the freezing takes place. The single experiment mentioned by Brücke in which the amputated thigh of a frog, after being submersed in distilled water and kept in a freezing mixture, was found to be in a state of rigor after thawing, is surely insufficient evidence for a claim that rigor is produced by cold; the rigor could have been due to the distilled water—water rigor.

Folin says further that "Brücke's experiments with cold as a means of producing rigor received, curiously enough, no attention from subsequent investigators and as far as I have been able to learn the discovery has been forgotten." Folin overlooked a paper by L. Hermann (*Pflüger's Archiv*, 1871, iv, 188) en-

5. By injections of such substances as chloroform, ether, caffeinbenzoate, etc.(26), directly into the muscles or intravenously, the muscles pass immediately into a state of strong rigor—chemical rigor.

Definition of Rigor.—Another puzzling question is: What constitutes rigor? Many deviations from the normal are met in rigor mortis: the muscles are stiff and of denser consistency than normal; their elasticity differs from that of normal muscles; they show an acid reaction, their irritability is lost, etc. Are all these changes essential parts of rigor and of every kind of rigor? Some believe that shortening of the muscles does not constitute an integral part of rigor. Many authors claim that the irritability of the muscle might persist even after the release from rigor(27). According to others the acid reaction is not indispensable for the development of rigor(28). Folin finally, as we have seen above, considers hardness and stiffness alone as the essential criteria of rigor.

We are apparently not yet ready for a final answer. A century of work has brought no decision between the contending theories. It has brought to light a great many single facts and that is what

titled: Die Erstarrung in Folge starker Kältegrade. This is the more regrettable as the knowledge of this paper might have been not without some influence upon Folin's method of investigation as well as upon his conclusions. As Folin did not return to this work again, as he intended to do, it might not be amiss to discuss here some of the essential points. Folin says that frog muscles which were cooled to -15° C. were found on thawing to have gone into rigor. The rigid muscles are perfectly translucent—not opaque—and apparently are not shortened. The first important conclusion drawn by Folin from these observations is that the only fundamental characteristic of rigor is the stiffness and hardness of the muscles and that opacity and shortening of the muscles, evolution of carbon dioxide, etc., are unessential incidents of the main phenomenon. On the implicit assumption that the muscles, while they are still frozen, are already in a state of rigor, extracts were prepared from them—before thawing—and compared with extracts made from normal muscles before rigor. It was found that the spontaneous coagulation as well as the heat coagulation was the same in both extracts, and that there was no difference with regard to the degrees of acidity as well as in the amounts of the total nitrogen. Since one of the extracts was made from muscles in rigor, Folin draws the conclusion that coagulation cannot be the cause of rigor mortis. It must be remembered that this conclusion is based upon the supposition that the muscles are in a state of rigor while they are still frozen. Now Hermann says that a thoroughly frozen muscle "verfällt nach dem Aufthauen einer beschleunigten Erstarrung," that is the rigor occurs *after* thawing; the freezing has only the effect of hastening

we still need and can accomplish at present: the uncovering of some more facts—the collection of more bricks for the erection of a structure at some future time.

THE RELATIONS OF CALCIUM AND MAGNESIUM TO RIGOR

Introductory.—The aim of our paper is the contribution of a few facts regarding the relations of calcium and magnesium to the phenomenon of rigor mortis. What are these relations? On *a priori* grounds our expectations may differ with our conceptions of the nature of rigor. From the point of view of the chemical theory we might expect that the mentioned alkali earths are capable of exerting a definite influence upon the development of rigor, i. e. that the character of the influence might be different for both substances. It is now well established that calcium salts hasten the clotting of blood. We do not know of a similar effect from magnesium salts; on the contrary, concentrated solutions of magnesium sulphate are employed to prevent or retard the clotting of blood. Do both substances behave in a similar manner towards the clotting of the muscle plasma and also towards the onset of rigor? That is, does

the onset of the rigor. Hermann says further that the thawed-out and not yet rigid muscle has a different appearance from the normal; it is translucent, and the (final) rigor of such frozen muscles differs from that of normal muscles by the *extreme degree of shortening and thickening* and by the exudation of a strongly acid serum. According to Hermann the rapid onset of the rigor does not depend upon thawing, or upon the degree and duration of the freezing, but upon the rapidity of the development of the latter. The essential points which are of interest to us here are, that according to Hermann the muscles, while they are still frozen, are not yet in a state of rigor, but that the freezing hastens greatly the onset of rigor, that the not-shortened translucent muscle is not yet in a state of rigor and that the real rigor which follows that stage is rather marked by an extreme shortening.

We offer of course no opinion as to which of the observations and views are the correct ones. But it seems to us that to uphold the conclusions of Folin the experiments ought to be repeated and the extracts ought to be made not from frozen muscles but after they were thawed out for some time, that is while they are in a state of undisputed rigor.

As to the question whether there is any difference between extracts made from muscles in rigor and from normal muscles, we may refer here to the recent investigations of Saxl (*Hofmeister's Beiträge zur Chemischen Physiologie*, 1906, ix, 1). He found that the proportion of "muscle plasma" obtainable from muscles in a state of postmortem rigor to that obtainable from fresh muscles is about 1:3.

calcium hasten the clotting of the plasma and also hasten the onset of the rigor, and does magnesium retard both? On the other hand, from the point of view of the contraction theory there are some reasons to expect that both ions might rather retard the development of rigor. It is well established, as we have mentioned above, that substances which increase muscular activity hasten the onset of rigor, while those which impair the contractions, curare, for instance, retard its onset. Now Loeb(29) has maintained that calcium exerts an inhibitory influence upon muscular contraction. A similar inhibitory influence Loeb ascribes also to the entire group of alkali earths except barium. We ourselves(30) have published several communications on the inhibitory effect of magnesium salts. But if calcium as well as magnesium is capable of inhibiting muscular contractions, rigor being in the nature of a contracture, we may expect that the effect of either ion would be in the direction of retardation of the development of rigor.

There are in the literature a few scattered statements bearing upon our question, more upon the effects of calcium than upon those of magnesium. Cavazani(31) reported that muscles poisoned by potassium oxalate do not pass into a state of rigor. As the oxalates precipitate calcium salts, Cavazani concludes from his observation that rigor is due to the presence of calcium salts in the muscles. However, neither Howell(32) nor Locke(33) were able to confirm the statement of Cavazani: muscles perfused with or kept in oxalate solutions passed into a state of rigor at least as early as the controls. From these experiments it could appear that calcium is of no importance in the development of rigor; but it must be remembered that potassium oxalate probably does not remove all the calcium from the muscles. Furthermore, Howell(34) himself found for heart muscle (strips of terrapin's heart) that a surplus of calcium chloride leads to rigor. Von Fürth(35), in studying the effect of many substances upon the clotting of muscle plasma and upon the development of rigor, found that the addition of calcium chloride, calcium nitrate or magnesium nitrate accelerated the clotting of a myogen solution, while the addition of magnesium sulphate did not exert such an influence. However, perfusion of the posterior extremities of frogs with ten per cent. solutions of calcium chloride or calcium nitrate did not hasten the onset of rigor.

A. Moore(36), working in J. Loeb's laboratory, tested the effects of various crystalloids upon rigor mortis by bathing the gastrocnemius muscles of frogs in solutions of these substances. For calcium chloride it was found that "a strong solution has at first a relaxing effect, but after a period varying from half an hour to one hour and a quarter, contraction begins, the lever reaching its maximum height in from three quarters to four hours." "In weaker solutions, $=n/2$, $=n/4$, contraction is slower in beginning and slower in setting, but as the strength of the solution is further decreased, rigor takes place more quickly." For magnesium chloride it is stated "that the effect is relaxing. It not only does not cause rigor, but actually prevents it." "Contrary to the above statement, however, rigor seems to be produced in the strength $=n/2$." For magnesium sulphate it is said that "no immediate effect was produced on the muscles immersed in the solution. A gradual rise began soon, however, indicating the approach of rigor, and the muscle assumed the usual rigor appearance. Rigor was noted in solutions stronger than $=n/4$."

In the extensive work of Overton(37) on the effect of different crystalloid solutions upon muscle and nerve the word rigor (Starre) does not occur, although frog muscles were kept in various solutions, sometimes for several days. We find, however, in the protocols (vol. 105, p. 220), that a frog muscle, kept in a solution of calcium chloride which is isosmotic with 0.7 per cent. solution of sodium chloride, about nine hours after removal from the body is "completely unirritable, shortened from thirty-eight to fifteen millimeters, opaque and very little plastic"; that can only mean that the muscle is in complete rigor about nine hours after death, which is for a frog muscle, kept at a temperature of 9° C., a very early onset.

Finally, Cushing(38) has found that the injection of Ringer's solution into the belly of a gastrocnemius muscle of a living frog may cause rigor which he ascribes to the presence of the calcium ion in the solution and compares this rigor with the calcium rigor of a strip of heart muscle described by Howell.

According to Moore, then, magnesium chloride as well as calcium chloride has more or less a relaxing effect upon the rigor of frog

muscles. These observations would seem to support, as argued above, the contraction theory of rigor. Moore, however, leans rather towards the chemical theory.

EXPERIMENTAL STUDIES BY THE WRITERS.

Method.—We made no observations upon the effect of bathing single muscles in the salt solutions. Our studies were made by injecting the solutions into living animals. The observations were made at first incidental to other experiments with magnesium and calcium salts. Injections were made into various species: dogs, cats, rabbits, guinea-pigs, rats and frogs. In the studies devoted directly to the subject of rigor mortis the most extensive observations were made upon rabbits. We experimented, however, also upon cats and frogs. In mammals the injections were made, at least in the main study, nearly exclusively intravascularly. In the frogs injections were also made into the lymph sacs. The intravascular injections in mammals were carried out by two methods: the intravenous and the intra-arterial. We shall report first the results we have obtained by intravenous injections.

Intravenous Injections of Calcium and Magnesium Salts.—The method of intravenous injection needs no special description. The solutions were permitted to run slowly into the external jugular vein from a burette arranged on the principle of a Marriotte's flask. Before beginning the infusion the animals were provided with a tracheal cannula and a cannula in the left external jugular vein. This, as well as any other operative procedure which may be mentioned later, was carried out under ether anesthesia. Usually two animals were studied at the same time, one for a magnesium and one for a calcium salt, the animals serving as controls for one another. In the magnesium animals artificial respiration had to be started pretty soon after beginning the infusion. But with artificial respiration and slow infusion a fairly good quantity can be introduced before the heart stops beating, the quantity, of course, depending upon the concentration of the solution.

In each double experiment the same molecular concentration of both salts was employed, and as nearly as possible the same quantity was infused into each animal. In the calcium animals the

respiration was rarely impaired during the entire time of the infusion, but they frequently received artificial respiration, nevertheless, in order to have similar conditions in both. The magnesium animals usually died from the infusion; the calcium animals mostly had to be killed at the end of the infusion, the animals being killed by opening the thorax. The solutions were employed as indicated above according to molecular concentrations, the weakest solution used being $= m/8$ and the strongest $= m/1$. The time elapsing between the death of the animal and the onset of rigor in the various parts of the body was taken as the main unit of comparison between the effects of the salts. The time of death is well marked, of course, by the final standstill of the heart. As to the appearance of rigor a distinction must be made between the fully developed state and its beginning, and between the rigor of the whole animal and that of the various parts. There is not the slightest doubt about the presence of rigor when it is in an advanced state, but its exact beginning is not always above dispute. Other difficulties with which one is confronted in this kind of observation are caused by variations in the time of onset of rigor in the individual animals, even under apparently exactly the same conditions, and by some variations in the order with which the rigor appears in the several parts of the body. However, the results obtained in our experiments were of such a palpable degree as to be in every instance above any doubt.

We shall now report our results, illustrating each series by abbreviated protocols of a few of the experiments.

ABBREVIATED PROTOCOLS OF SOME EXPERIMENTS.

Series I. Intravenous Infusions of the Salts in Molecular Solutions.

EXPERIMENT 1.—Magnesium chloride. Female rabbit, 1060 grams. Ether; tracheotomy; cannula in external jugular vein, connected with burette which contained magnesium chloride in $M/1$ solution.

11.55 a. m. Infusion begun. When 1.5 c.c. ran in, spontaneous respiration disappeared; started artificial respiration.

11.59 p. m. 4.5 c.c. ran in; animal dead. Thorax opened; left ventricle moderately contracted, right dilated and soft.

2.10 p. m. Left ventricle relaxed again. No stiffness in jaws, neck or legs.

3.55 p. m. Jaws locked; perhaps slight stiffness in neck; extremities still soft.

4.40 p. m. Neck stiffer; some stiffness of front and hind legs.

6.00 p. m. Neck stiff; rigidity of front and hind legs has progressed, but is far from being complete. (No further note on heart.)

The next day at 10.30 a. m. the animal is quite rigid.

In this experiment with magnesium chloride the jaws became rigid about four hours after death and at that time the rigidity of the neck just began. The rigidity of the legs began about four hours and forty minutes after death. It was not observed at what time the rigor attained its maximum, it was far from it six hours after death; it was found, however, to be complete next morning.

EXPERIMENT 2.—Magnesium sulphate. Female rabbit, 1140 grams. Same preparation as in previous experiment; burette was filled with magnesium sulphate in *M/1* solution.

12.21 p. m. Infusion begun; artificial respiration started soon after.

12.30 p. m. Heart stopped, 6.5 c.c. infused.

2.10 p. m. No stiffness of any part of body, heart soft, moderately dilated.

3.55 p. m. Jaws locked; neck soft.

4.40 p. m. Neck soft; front legs soft; hind legs slightly stiff at hips.

6.00 p. m. Neck getting stiff; stiffness in all four legs, increased in hind legs.

Next day at 10.30 a. m. legs and neck are rigid.

In this rabbit (magnesium sulphate) the jaws were locked after about three hours and a half, while rigidity of the neck did not begin until five and a half hours after death. Also the rigidity of the legs was more retarded in this animal than in the magnesium chloride rabbit.

EXPERIMENT 3.—Calcium chloride. Female rabbit, 1200 grams. Burette filled with calcium chloride in *M/1* solution.

12.48 p. m. Infusion begun.

12.54 p. m. Respiration good, but artificial respiration started.

12.57 p. m. Heart stopped. Infusion stopped, 5 c.c. infused.

1.10 p. m. Heart contracted, left ventricle more than right.

2.10 p. m. Neck quite stiff; rigor in hind legs, good stiffness on extension at knee.

2.45 p. m. Full rigor in hind legs.

3.55 p. m. Rigor quite marked in front legs.

4.40 p. m. Complete rigor in entire body (no note on jaws).

Next day at 10.30 a. m. the body is less stiff and the neck is soft.

In this animal (calcium rabbit) the neck was quite rigid one hour and fifteen minutes after death, and at that time the rigidity of the hind legs also was fairly established. In the front legs a marked rigidity was present about three hours after death. About four hours after death the entire rigor was complete. But next morning the release from rigor had already begun.

We shall add one more protocol of a calcium experiment, carried out with a *M/1* solution.

EXPERIMENT 4.—Calcium chloride. Female rabbit, 2000 grams. Same preparation; right sciatic nerve cut at exit from pelvis; burette filled with calcium chloride in *M/1* solution.

12.20 p. m. Infusion begun, given very slowly; artificial respiration started.

12.39 p. m. Convulsions appeared.

12.43 p. m. Infusion stopped, 8 c.c. infused. Artificial respiration stopped; no convulsions; heart continues beating freely for one minute. Soon after heart dilated and flabby.

1.00 p. m. Left ventricle contracted, right soft.

1.30 p. m. Neck quite rigid; jaws can just be opened with force; front legs show slight stiffness on flexion; hind legs some resistance on flexion; definite rigor on extension at hips; right leg stiffer than left.

2.00 p. m. Jaws locked; neck more stiff; marked stiffness of front legs on flexion; rigidity of hind legs increased.

5.15 p. m. Stiff as a board everywhere; right hind leg fully extended; left hind leg more flexed.

Next day at 10.00 a. m. neck is soft, jaws can be opened and the entire animal shows good relaxation—more so than another rabbit which received on the previous day only 4 c.c. of calcium chloride in *M/1* solution.

In this (calcium) animal the rigor was everywhere fairly established about forty-five minutes after death. The maximum rigor of the entire animal was attained in four and a half hours. Next morning, however, the release from rigor had already begun and was considerably advanced.

In this series with intravenous infusion of molecular solutions the onset of rigor in the animals receiving magnesium chloride and magnesium sulphate was delayed by a few hours in comparison with the onset of rigor in the calcium animals. In the latter the onset as well as the complete development of the rigor appeared quite early and the earlier the larger the infused quantity was. The release from rigor appeared earlier in the calcium than in the magnesium animals.

Series II. Intravenous Infusions of M/2 Solutions.

EXPERIMENT 4.—Magnesium chloride. Female rabbit, 1700 grams. Preparation same as in previous experiments; burette filled with magnesium chloride in *M/2* solution.

11.09 a. m. Infusion begun.

11.10 a. m. Respiration shallow; artificial respiration started.

11.20 a. m. 19 c.c. ran in; occasional heart beats; artificial respiration stopped; animal dead (thorax not opened).

12.42 p. m. No stiffness anywhere.

12.50 p. m. No definite stiffness.

5.00 p. m. Moderate, but definite stiffness of hind and front legs, more in hind legs.

Next day, 11.00 a. m., animal stiff; no retraction of head, and legs not extended; right ventricle of heart dilated and soft, left ventricle moderately contracted. No relaxation of body set in during the day.

Five and a half hours passed before any sign of rigor appeared. The complete rigor observed next day was of a flexion type. There was no relaxation next day.

EXPERIMENT 6.—Calcium chloride. Female rabbit, 1800 grams. Burette filled with calcium chloride in $M/2$ solution.

12.01 p. m. Infusion begun; no artificial respiration.

12.14 p. m. 19 c.c. ran in; infusion stopped. Irregular respiration; feeble heart beats; a few convulsive struggles.

12.16 p. m. No heart beats felt; dead, thorax not opened.

12.42 p. m. Definite stiffness of hind legs; front legs soft.

1.15 p. m. Definite stiffness of front legs also.

2.15 p. m. Entire animal rigid.

5.00 p. m. Animal lying with head retracted, all four legs strongly extended and with marked lordosis of back.

Next day at 11.00 a. m. the animal is in the same position, but is not so stiff; both ventricles are strongly contracted.

1.30 p. m. No longer as rigid as on previous day; especially front legs soft again.

Twenty-six minutes after death of this calcium animal definite rigor was present; the animal showed later a strong rigor in extreme extension, but the release from rigor had already begun early on the following day.

Experiments on cats with $M/2$ solutions brought out the same differences between calcium and magnesium chloride observed in rabbits: early onset, and development of rigor of an extension type with a comparatively early release, in the calcium animal, and late onset, and development of rigor of a flexion type, with a late release in the magnesium animal. The left ventricles showed the same differences as the skeletal muscles.

Series III. Intravenous Infusions of $M/4$ Solutions.

EXPERIMENT 7.—Magnesium chloride. Male rabbit, 1180 grams. Same preparation; burette filled with magnesium chloride in $M/4$ solution.

11.34 a. m. Infusion begun, running slowly.

11.38 a. m. 5 c.c. infused; respiratory struggles; artificial respiration started.

11.50 a. m. Stopped infusion, 26 c.c. infused; heart just perceptible; stopped artificial respiration.

11.52 a. m. Thorax opened; heart not beating.

1.00 p. m. All parts of body flaccid, heart soft.

1.55 p. m. Same.

2.55 p. m. Jaws cannot be opened; neck and extremities soft.

3.50 p. m. Same.

5.30 p. m. Rigor beginning in hind and front legs, neck still soft.

Next day all parts in rigor; unchanged the entire day.

In this animal rigor began in the jaws and muscles after three hours and in the legs after five and a half hours, while the neck was still soft. There was no relaxation next day.

EXPERIMENT 8.—Calcium chloride. Female rabbit, 1690 grams. Same preparation; burette filled with calcium chloride in $M/4$ solution.

12.09 p.m. Infusion begun.

12.23 p. m. 15 c.c. infused. Heart slightly irregular; started artificial respiration.

12.30 p. m. Stopped infusion; 26 c.c. infused; stopped artificial respiration; heart good, but no respiration—apnoea.

12.32 p. m. Breathes; opened thorax; death under signs of asphyxia.

1.25 p. m. Both ventricles contracted; body flaccid.

1.55 p. m. Jaws and neck rigid.

2.55 p. m. Marked rigidity of entire body.

3.50 p. m. Extreme rigor.

Next day at 12 m., neck and front legs are soft.

In this (calcium chloride) animal the rigor began in the jaws and the neck after one hour and twenty minutes and in the rest of the body after two hours and twenty minutes. The rigor attained its maximum, however, in three and a half hours. The release began early the following day.

EXPERIMENT 9.—Magnesium nitrate. Male rabbit, 2450 grams. Same preparation; burette filled with magnesium nitrate in $M/4$ solution.

1.08 p. m. Infusion begun.

1.18 p. m. 13 c.c. infused; respiration shallow; artificial respiration started.

1.31 p. m. 32 c.c. infused; heart quite weak; stopped infusion for three minutes.

2.00 p. m. Stopped infusion, 51 c.c. infused; stopped artificial respiration; no reaction whatsoever.

2.05 p. m. Thorax opened; heart beats; a good deal of bleeding.

2.15 p. m. Heart stopped beating; everything soft; blood clots poorly.

3.50 p.m. Jaws slightly stiff, everything else flaccid.

5.00 p. m. Jaws locked; every other part of body quite soft. Both ventricles soft; right ventricle contracts on touch.

6.00 p. m. No change.

8.30 p. m. Slight rigor in legs, not pronounced; very slight stiffness in neck, if any; left ventricle rather strongly contracted.

Next day, rabbit is very stiff and rigor is of a flexion type.

In this (magnesium nitrate) animal there was even after six hours practically no rigor in extremities and neck, the jaws however

already began to show stiffness one hour and thirty minutes after death.

EXPERIMENT 10.—Calcium nitrate. Female rabbit (weight ?). Usual preparation; burette filled with calcium nitrate in $M/4$ solution.

10.50 a.m. Infusion begun.

11.10 a. m. 15 c.c. infused so far; respiration poor; inspiratory tonus; started artificial respiration.

11.25 a. m. Stopped infusion, 25 c.c. ran in. Thorax opened, no heart beats.

11.08 a. m. Jaws locked; neck slightly stiff; moderate but definite stiffness in front legs.

12.10 p. m. Some stiffness in hind legs; left ventricle strongly contracted.

12.50 p. m. Front legs extended; stand out like sticks, hind legs very stiff, but still flexed. (Not observed any further.)

This animal received of calcium nitrate $M/4$ about one half of the quantity which the animal in the foregoing experiment received of magnesium nitrate. The contrast between the two animals was very striking. While in the magnesium animal there was no rigor even six hours after death, there was in the calcium animal a definite rigor already thirty minutes after death.

EXPERIMENT 11.—Calcium acetate. Male rabbit, 1850 grams. Same preparation; burette filled with calcium acetate in $M/4$ solution.

2.15 p. m. Infusion begun.

2.32 p.m. 10 c.c. infused; lid reflex active; respiration good, but heart cannot be felt; convulsions set in. Artificial respiration started and thorax opened. Heart beats extremely rapidly, left ventricle almost in complete tetanus; very slight diastolic relaxations. (Artificial respiration, though strong, produces no apnoea.) Six cubic centimeters more given.

2.40 p. m. Heart stopped; animal dead. Heart soon became soft.

2.50 p. m. Heart beginning to show rigor, progressing from base to apex; was completed in three minutes; right ventricle remains soft, auricles pale.

3.05 p. m. Neck, jaws and four legs in good rigor. The rapid progress could be directly perceived; the legs were seen moving, shortening, sometimes in short starts.

4.30 p. m. No change; complete rigor.

Next day at 3 p. m., the neck was soft, also jaws and legs a good deal softer.

The development of the rigor in this (calcium acetate) animal was a striking spectacle. Twenty-five minutes after death the rigor was complete, and the progress was a matter of ocular perception.

In this series the magnesium nitrate had the most delaying, and calcium acetate a most accelerating effect upon the onset and development of rigor.

Series IV. Intravenous Infusion of M/8 Solutions.

EXPERIMENT 12.—Magnesium chloride. Female rabbit, 1445 grams. Same preparation; right sciatic nerve cut; burette filled with magnesium chloride in M/8 solution.

11.00 a. m. Infusion begun.

11.01 a. m. Started artificial respiration.

11.05 a. m. Infusions stopped; 30 c.c. infused.

11.07 a. m. Thorax opened; heart beats occasionally.

12.50 p. m. Left ventricle slightly contracted; everything soft otherwise.

3.20 p. m. Both hind legs moderately stiff, right more than left; front legs show beginning of rigor.

4.00 p. m. Both hind legs stiff, now left more than right; front legs stiffer than before; left ventricle well contracted, right still dilated and soft.

5.30 p. m. All legs very stiff; no difference between hind legs.

Next day at 2.30 p. m., front legs are soft, hind legs stiff.

In this animal with a solution of magnesium chloride nearly isosmotic with a "physiological" sodium chloride solution, the rigor began earlier than with the more concentrated solutions—about three hours after death, attaining its maximum in about six hours and showing already in the afternoon of the next day a distinct beginning of release from rigor.

EXPERIMENT 13.—Calcium chloride. Female rabbit, 1370 grams. Same preparation; right sciatic cut; burette filled with calcium chloride in M/8 solution.

11.27 a. m. Infusion begun.

11.37 a. m. Stopped infusion; 30 c.c. infused; convulsions; thorax opened; heart still beating.

12.40 p. m. Neck stiff, front legs, especially right, stiff; stiffness of hind legs beginning; no difference between right and left.

12.50 p. m. Right and left ventricles strongly contracted.

1.20 p. m. Left hind leg definitely stiffer than right.

5.30 p. m. Legs in full rigor, no difference between hind legs. Next day in afternoon all legs more or less soft again.

In this (calcium) animal the onset of rigor occurred also pretty early, about one hour after death, but the maximum was not attained until about six hours after death. The onset of rigor in the right ventricle occurred unusually early, after about one hour and ten minutes.

Transfusion with Sodium Chloride.—We append here an abbreviated protocol of one of the experiments made with transfusion of sodium chloride in molecular solution. It may serve as a control experiment to the foregoing observations.

EXPERIMENT 14.—Sodium chloride. Male rabbit, 2039 grams. Same preparation; burette filled with sodium chloride in $M/1$ solution.

12.43 p. m. Infusion begun.

12.55 p. m. Stopped infusion; 7.5 c.c. infused; animal showed not the slightest reaction; heart, respiration, lid reflex, etc., unchanged.

12.57 p. m. Killed by clamping the trachea. When the convulsions due to asphyxia were over, the thorax was opened; heart beating feebly.

1.10 p. m. Heart beating slightly.

1.45 p. m. Left ventricle well contracted; everything else flaccid.

2.05 p. m. Jaws show good stiffness; some stiffness on extension at elbow and thigh.

3.20 p. m. Neck rigid; front legs stiff on extension and flexion; hind legs very rigid at hip on extension; beginning rigor in other parts.

3.50 p. m. Practically stiff all over.

Next day, 3.30 p. m., neck soft, other parts also less rigid.

In these cases the rigor set in later than in the calcium and earlier than in the magnesium animals.

The sodium chloride animals were very little affected by the transfusion, and at the end of it had to be killed. The killing was done either by clamping the trachea or by free opening of the thorax, and the asphyxia brought on the usual antemortem convulsions. Now since any kind of convulsion hastens the onset of rigor, and since magnesium animals die without convulsions, the question arose, whether it was not these convulsions which made the rigor appear earlier in the sodium chloride animals than in the magnesium rabbits. Furthermore the very same question had to be met with regard to the accelerating effect of calcium, since the calcium salts did not paralyze the animal and frequently indeed convulsions appeared either in the course of the transfusion or shortly before the death of the calcium animals. In other words, we were confronted with the question whether the difference in the time of onset of the rigor between the calcium and magnesium animals was not due simply to the fact that magnesium paralyzes the animal and calcium does not.

Experiments with Curare in Addition to the Salts.—We have therefore carried out a few experiments in which convulsions were prevented by the previous administration of curare. We shall illustrate the results by the following two protocols.

EXPERIMENT 15.—Calcium chloride; curare. Male rabbit, 1907 grams. Usual preparation; burette filled with calcium chloride in $M/1$ solution. Curarin injected until spontaneous respiration was abolished; started artificial respiration.

12 m. Infusion begun.

12.11 p. m. Heart not palpable; stopped infusion; 7.5 c.c. ran in; stopped artificial respiration; no convulsions. Thorax opened; occasionally a heart beat.

12.50 p. m. Heart moderately contracted; jaws and legs show beginning stiffness.

1.20 p. m. Jaws locked; neck rigid; legs rigid and extended.

2.45 p. m. Rigor nearly complete, of an extension type. Next day very little softening was present.

Curare, which usually retards the onset of rigor, here exerted no influence whatsoever. Although no convulsions occurred, the rigor developed as early as is usual with such a dose of a molecular solution of calcium chloride. This experiment teaches therefore in the first place that the acceleration of the onset of rigor by the transfusion of calcium salts is not due to an antemortem appearance of convulsions. But it teaches us also that the delaying effect of curare is completely wiped out by the accelerating effect of calcium.

EXPERIMENT 16.—Magnesium chloride; curare. Female rabbit, 1320 grams. The usual preparation; burette filled with magnesium chloride in *M/1* solution. Curare given until effective; started artificial respiration.

12.35 p. m. Infusion begun; given very slowly.

12.50 p. m. Stopped infusion, 7 c.c. infused; stopped artificial respiration. No convulsions; thorax opened; no heart beats.

2.05 p. m. Everything perfectly soft.

2.45 p. m. Jaws stiff, everything else flaccid.

4.45 p. m. Except in jaws no stiffness in any part, including heart. Animal was not seen until next day at 4 p. m., when the following note was made: Some rigor of legs present, but less than in the calcium chloride rabbit.

In this and in other experiments in which magnesium salts and curare were employed at the same time, the delay in the onset of the rigor was greater than in animals which had either magnesium alone or curare alone. The retardation of rigor mortis after magnesium salts would therefore seem to be in a degree due to some other factor besides the capability of paralyzing nerve and muscle.

Experiments with Magnesium Salts and Strychnine.—That, however, the paralyzing effect of magnesium salts is an essential factor in the delay of rigor can be seen from the following two experiments, in which, besides magnesium salts, strychnine was administered.

EXPERIMENT 17.—Magnesium chloride and strychnine. Female rabbit, 1940 grams. Usual preparation; burette filled with magnesium chloride in *M/2* solution.

2.27 p. m. Infusion begun; given slowly.

2.35 p. m. 3 c.c. infused so far; respiration slow and labored; started artificial respiration.

2.38 p. m. 9 c.c. infused so far; no corneal reflex; animal limp.

2.40 p. m. Injected intramuscularly 1.5 milligrams strychnine nitrate—more than a minimum fatal dose.

2.54 p. m. Stopped infusion; 18 c.c. infused.

2.55 p. m. Artificial respiration stopped temporarily, heart beating; artificial respiration resumed.

2.58 p. m. Stopped artificial respiration; no convulsions.

3.00 p. m. Animal dead.

3.48 p. m. and 4.25 p. m. All parts perfectly limp.

5.15 p. m. Jaws stiff now; all other parts flaccid.

Animal was not seen until next day at 10.30; then all parts were rigid.

There were no strychnine convulsions; the magnesium salts as well as the artificial respiration inhibited them, and there was apparently the same delay in rigor as seen after the administration of magnesium salts alone.

EXPERIMENT 18.—Magnesium sulphate and strychnine. Female rabbit, 1940 grams. Usual preparations; burette filled with magnesium sulphate in $M/2$ solution.

11.09 a. m. Injected subcutaneously one milligram of strychnine nitrate, 0.5 milligram per kilo being only a toxic dose.

11.16 a. m. Animal hyperesthetic.

11.19 a. m. Convulsions; started artificial respiration and began infusion of magnesium sulphate very slowly.

11.22 a. m. Convulsions stopped; no hyperesthesia.

11.24 a. m. On stopping artificial respiration tremor of chest began again; artificial respiration resumed.

11.35 a. m. No tremor anywhere.

11.42 a. m. Infusion stopped; 30 c.c. ran in; stopped artificial respiration; no convulsions or tremor followed.

11.48 a. m. Heart still beats occasionally.

12.15 a. m. Definite stiffness of hind and front legs.

12.55 a. m. Stiff as a board.

Next day at 5 p. m., there is no beginning of release from rigor.

Although this animal received a very large dose of magnesium sulphate, the rigor was not delayed by it; the onset and development were as much accelerated, as if strychnine alone had been administered. The accelerating effects of a tetanus apparently cannot be perceptibly modified by the subsequent administration of magnesium salts.

We have made a few experiments on the onset of rigor under so-called normal conditions of death, but we shall not enter into

a discussion of these experiments nor draw any conclusions from them. From our own studies and from a study of the literature we believe that every mode of killing employed by various writers, for instance, pithing, hanging (asphyxia), exsanguination, etc., has a specific relation to the onset of rigor and has to be studied separately in a longer series of observations.

Conclusions Drawn from the Infusion Experiments.—Our experiments with the intravenous infusion of calcium and magnesium salts appear to have quite firmly established the following facts. In the first place, it seems quite certain that calcium salts hasten and magnesium salts retard the onset of rigor mortis in skeletal muscles. These effects appear to increase with the quantity of the salts introduced into the circulation; the degree of the molecular concentration in which the solutions are employed seems to be of lesser importance. The accelerating effect of the calcium salts is apparently independent of the state of contraction of the muscles; there is a strong acceleration even when antemortem convulsions are prevented by means of curare. The delay due to magnesium is apparently caused, in the first place, by the paralyzing effect of these salts upon the nervous system and the muscles. Besides the paralyzing effect, the magnesium salts may, however, contain another factor which aids in a smaller degree in the delay of onset of the rigor. The delay following magnesium plus curare seemed to be greater than that following either magnesium alone or curare alone.

Another striking difference, which was frequently observed in these experiments and which deserves to be recorded, consisted in the difference of the type of rigor which followed each of the two kinds of salts. After infusion with calcium salts, especially after larger quantities, the preponderance of extension was very manifest. Even when the animals soon after death were placed in a position in which flexion prevailed, namely, with the head bent forward, the abdomen concave and all the extremities flexed, they were found later in a strong position of opisthotonus: the head strongly drawn backward with a pronounced lordosis and the extremities stretched out. The position of the magnesium animals, on the other hand, was always that in which flexion prevailed, or perhaps more correctly, the animals remained in the position in which they were placed originally.

As to the degree of rigidity which the muscles finally attained, there seemed to be no difference between calcium and magnesium, both animals were at one time or another stiff as a board, only that the maximum stiffness was attained in the magnesium animals much later than in the calcium animals.

The release from rigor appeared as a rule definitely earlier in the calcium animals than in the magnesium animals. There were only a few exceptions in which the rigor in the calcium animals lasted as long or even longer than in the magnesium animals and in these cases the quantity of calcium salts used was greater in proportion than that of the magnesium control. The earlier release from rigor in the calcium animals seems to be due to the earlier development of rigor and occurs in all cases in which the rigor for any reason sets in early (Bierfreund). We have, however, to state that on account of the high temperature of the season in which most of the experiments were carried out not all of the animals were kept until the release from rigor was completed.

The action of the salts upon rigor was caused, at least in the main, by the cations, that is, by the calcium and magnesium parts of the compounds. As regards the anions it is probable that they too exert some effect. Calcium acetate, for instance, seemed to hasten the onset of rigor more than any of the other calcium compounds. Our experiments were in this regard not sufficiently numerous and varying to permit the giving of precise data. We believe, however, that our experiments justify the general statement that the effect which the anions may have is insignificant in comparison with the effects which the cations of the salts exert upon the onset of rigor.

As regards the order in which the several parts of the body enter into rigor, the rigidity of the jaws appeared first in our experiments and usually far ahead of the stiffness of the other parts. The neck was the next part in which stiffness appeared. In a few cases, however, the neck was the last part in which the rigor set in. Of the extremities, in the majority of our experiments, the hind legs became rigid before the front legs. This differs from the so-called Nysten system, according to which the arms become rigid before the legs, For rabbits, however, our observation agrees with those of previous observers(14).

Cutting the sciatic nerves, which is said to retard the development of rigor(39), produced in our experiments no definite results. Sometimes there was a slight retardation of the onset on the side on which the nerve was cut, but nearly as often the retardation was on the opposite side. Furthermore, even in animals with both sciatic nerves intact, sometimes the right and sometimes the left leg became stiff first; the same occurred also in the front legs.

Finally we made incidentally some notes on the onset of rigor in the heart. The observations were not so extensive and precise as those made on the skeletal muscles; but they were sufficiently numerous to permit the general statement that calcium hastens and magnesium retards the development of rigor of the left ventricle. The observations upon the right ventricle were insufficient to warrant any positive statement.

Experiments with Intra-arterial Injections.—In the foregoing experiments with intravenous infusions, the salts, even when given in molecular concentration, reached the tissues in a dilute state and reached them always in company with some blood, which according to von Fürth(26), prevents the coagulation of the muscle plasma and interferes also with the development of muscle rigor. In the experiments which we are now going to record the injections were made into the arteries towards the periphery. In these experiments the muscles of that peripheral part towards which the injections were made, came into intimate contact with a more concentrated solution of the injected salts and probably also without a great admixture of blood.

As mentioned above, such experiments were already made by von Fürth. He tested a large number of substances in mammals as well as in frogs. However, as far as the substances employed in our experiments are concerned, he made only two experiments in frogs, one with calcium chloride and one with calcium nitrate; at least these are all that he records and it is not indicated that more experiments were performed than recorded. Von Fürth has seen no accelerating effects from injections with calcium salts. We have made such injections with calcium and magnesium salts in rabbits and frogs. We shall report first our experiments on rabbits.

Intra-arterial Injections in Rabbits; Method.—The animals had

morphin and ether; laparotomy was made. The abdominal aorta was ligated always beneath the renal arteries, and a cannula was tied in its peripheral end. In some experiments the inferior vena cava also was ligated, in others again the cannula was tied in one of the common iliac arteries. When the cannula was in the aorta, one of the common iliacs was clamped during an injection in order to direct the solution into one limb, using the other as a control. The injection was made with a syringe and usually quite rapidly—not less than six or seven cubic centimeters in a minute. In these experiments calcium chloride only was tested, while of the magnesium salts the chloride and the sulphate were investigated. Only $M/8$ and $M/1$ solutions were used.

In these experiments the animals usually died during the injection or soon after it, even when the inferior vena cava was clamped—a proof of the sufficiency of the collateral circulation. When the injections were made into the aorta, with one common iliac clamped, the tissues of both sides of the lumbar region were seen to be invaded by the liquid and to become blanched. Probably also the lumbar section of the spinal cord came into intimate contact with the solutions, a point which may have to be taken into consideration when analyzing the results.

We shall again illustrate our results with a few greatly abbreviated protocols.

Series I. Intra-arterial Injections into the Hind Legs of Rabbits; $M/8$ Solutions.

EXPERIMENT 19.—Calcium chloride. Female rabbit, 1350 grams. Preparation as stated above; abdominal aorta ligated. Cannula tied in left common iliac artery.

- 12.30 p. m. Injected 20 c.c. calcium chloride of $M/8$ solution into left leg.
- 12.34 p. m. Thorax opened, death; no immediate stiffness.
- 12.40 p. m. Left leg shows some resistance.
- 1.30 p. m. Distinct stiffness in left leg, right flaccid.
- 1.55 p. m. Left leg stiff in all joints, right leg soft.
- 3.25 p. m. and 4.20 p.m. Left leg like a board, held extended; some resistance in right leg.

In the calcium leg the rigor began about ten minutes after injection and in the control leg not till after three hours.

EXPERIMENT 20.—Magnesium sulphate. Female rabbit, 1450 grams. Usual preparation; cannula in aorta; left iliac clamped.

11.55 a. m. Injected 30 c.c. magnesium sulphate in *M/8* solution (into right leg). Had slight convulsions after injection.

12.00 m. Thorax opened, heart still beating.

4.00 p. m. Front legs and left hind leg stiff; right leg flaccid.

The magnesium leg was flaccid, while the other parts were in full rigor.

EXPERIMENT 21.—Calcium chloride in one leg and magnesium chloride in the other. Female rabbit, 1120 grams. Usual preparation; cannula in aorta.

11.30 a. m. 13 c.c. *M/8* magnesium chloride injected into left leg (right iliac clamped).

11.34 a. m. Struggles.

11.35 a. m. 13 c.c. *M/8* calcium chloride injected into right leg (left iliac clamped). Thorax opened, heart still beats.

11.50 a. m. Right leg (calcium) stiffer than left.

12.40 p. m. Left leg soft; good resistance in right.

3.20 p. m. Right leg very stiff; some stiffness in left.

In this experiment the difference between the calcium and the magnesium was striking enough, but the acceleration of the rigor in the calcium leg and the delay in the magnesium leg were not so striking as in the other experiments in which larger doses were injected.

In these and other experiments of this series the calcium was distinctly accelerating and the magnesium delaying on the onset of rigor, the injected quantity being a noticeable factor. There was no perceptible effect immediately after injection.

Series II. Intra-arterial Injections of M/1 Solutions.

EXPERIMENT 22.—Calcium chloride. Male rabbit, 1410 grams. Usual preparation; cannula in aorta; left common iliac clamped.

10.30 a. m. Injected 30 c.c. *M/1* solution (into right leg); animal died during injection.

10.33 a. m. Right leg definitely stiff at hip.

10.35 a. m. Right leg stiff at all joints.

11.30 a. m. Right leg very stiff; slight stiffness in left leg as well as in front legs.

This greatly abbreviated protocol shows that in the injected leg the rigor began two or three minutes after injection, and was not followed by any temporary relaxation, while in the other parts of the body the rigor began about one hour after death.

Out of four experiments with calcium chloride, *M/1*, only in one was there a brief relaxation intervening between the primary contraction and the final rigor.

EXPERIMENT 23.—Magnesium sulphate. Female rabbit, 1250 grams. Usual preparation; cannula in aorta; left common iliac artery clamped.

11.35 a. m. Injected (into right leg) 30 c.c. $M/1$ magnesium sulphate; animal died during injection.

11.38 a. m. Definite stiffness of right leg at hip, left leg soft.

12.05 p. m. Both legs soft at all joints.

2.00 p. m. Both hind legs soft; slight stiffness of neck and front legs.

3.30 p.m. Some stiffness at hip in both legs; more on right side.

Three minutes after the injection of magnesium sulphate the injected leg became stiff, but softened soon, to become rigid again. Rigidity occurred even later than in the other parts of the body. The same occurred in two other experiments with $M/1$ magnesium sulphate.

In two experiments with $M/1$ magnesium chloride (30 c.c.) the leg into which the injection was made became stiff soon after the injection, the stiffness developing into complete rigor without an intervening relaxation. The injected leg behaved in these experiments rather like the leg in the majority of the calcium experiments. With regard to the other parts of the body there was no difference in the behavior towards the onset of rigor between the magnesium sulphate and magnesium chloride animals. In both cases the rigor developed in the rest of the body later than in the calcium animals, but not as late as in the animals which received the magnesium salts intravenously.

While in the intravenous injections the degree of concentration of the solutions affected but little the final results, in the intra-arterial injections the degree of concentration was quite an important factor. When the salts were injected in $M/10$ solutions, there was in the intra-arterial injections the same difference between the magnesium and calcium salts as in the intravenous injections, the former uniformly delaying, the latter accelerating the onset of rigor. In either case the cation was the main factor and the anion was of very little importance. It was very different, however, when in the intra-arterial injections the salts were given in $M/1$ solution. Here the calcium chloride as well as both magnesium salts brought on a distinct stiffness almost immediately after injection. In the magnesium sulphate animal the stiffness gave way again, the real rigor developing much later. In most of the calcium animals the early stiffness went over into complete rigor without any temporary relaxation.

The same occurred also in the magnesium chloride animals. The

early stiffness in all these cases was apparently not rigor, but simply a real contraction of the muscles, caused by the stimulation of the concentrated solutions. It was neither a specific calcium nor magnesium effect.

The rapid injection of a $M/1$ solution had the effect of a so-called "salt action," an osmotic action, perhaps. And here we found that the anion is a significant factor, the magnesium chloride acted more like calcium chloride and less like the magnesium sulphate. That in the cases of both chlorides the primary stiffness passed over directly into rigor might have been due to the greater contraction which they have primarily produced and finds its analogue in the direct transition of a strychnine tetanus into rigor, the development of which, as we found above, magnesium salts cannot suppress or delay. The same conditions probably obtain in all cases of so-called chemical rigor (see von Fürth), for instance, after injection of chloroform, ether, caffeine, sodium mono-brom-acetate, quinine, anti-pyrin, etc.; these substances cause primarily strong contractions, which hasten the onset of rigor and into which the contractions pass without any intervening relaxation. These chemical rigors are work-rigors (*Arbeitsstarre*) as Santesson (40) properly terms them. The rigor which follows nearly immediately after the injection of calcium and magnesium chloride belongs therefore to the group of work-rigors and two stages must be distinguished: the stage of simple tonic contraction and the stage of actual rigor. If the tonic contraction is moderate as in most of the cases when magnesium sulphate is injected in $M/1$ solution a longer or shorter intermission of relaxation follows between the stage of contraction and that of the real rigor.

From the production of this work-rigor must be distinguished the accelerating effect of the calcium salts upon the onset of rigor in the cases where only $M/8$ solutions were employed intra-arterially or when even $M/1$ solutions were injected intravenously. In these cases at least twenty or thirty minutes passed before the onset of rigor which was never preceded by a preliminary contraction. The effect here is apparently purely a chemical one confined to the cation calcium and is never caused by any anion combined with magnesium.

In the intra-arterial injections into one of the legs the effect of

the salts upon the other parts of the body was not so well pronounced as in the intravenous injections, although the entrance of the solution into the general circulation was prompt enough to prove fatal to the animal. The premature killing of the heart caused by the rapidity of the injection prevented the salts from satisfactorily reaching and saturating the tissues so as to bring them under the same degree of influence as in the slow intravenous injections with a prolonged efficient circulation.

The subcutaneous injections which were made on rabbits, guinea-pigs and rats were not numerous and we shall not speak of them in detail. It will suffice to say that the results of these observations agree with the main results obtained by the intravascular method, namely that calcium hastens and magnesium retards the onset of rigor.

Experiments on Frogs.—The injections were made in the lymph sacs and through the aorta. The rigor in frogs sets in very late after death, sometimes even as late as thirty-six hours. The interval varies with the species of frog, with their state of nutrition, and with the season of the year. Our studies were made on *Rana esculenta*, in the early spring and at the end of the summer, and some of the animals were in a rather poor state of nutrition. In all of the controls the rigor never appeared within the first twenty-four hours. As out of every twenty-four hours the animals can be under direct consecutive observation not more than ten, it is frequently impossible to note the onset of rigor if no artificial means are employed to hasten it. We made only two sets of experiments in which the animals received the injection of the salt solutions in the lymph sacs and then left them without further interference. Another drawback in the experiment is that if both frogs received the injection simultaneously, the calcium animal survived the magnesium animal by many hours, in fact, the exact time of their death could not be noted in our experiments; they were found dead in the morning. But even so, the calcium animals of our experiments were found already quite rigid, while the magnesium animals, although they died shortly after the injection in the middle of the previous day, were either still completely soft or showed only slight stiffness. We may therefore conclude even from these few, incom-

plete experiments that calcium hastens the onset of rigor in frogs. As to the delaying effect of magnesium salts these experiments gave no positive evidence.

In the experiments with transfusions through the aorta calcium chloride, magnesium chloride, magnesium sulphate and also sodium chloride were tested in $M/1$, $M/2$ and $M/10$ solutions. In the infusions with $M/1$ solutions all animals became very stiff in the course or at the end of the infusion and remained so for several days, while the control animals became rigid on the second day and were relaxed again about twenty-four hours later. The brain was destroyed in all animals. The cord was destroyed in one set and left intact in another set. In the animals with destroyed cords the rigidity was a little less strong than in the animals with cord intact. The early and persistent rigidity occurred not only in the calcium frog, but also in the animals which received magnesium sulphate, magnesium chloride or sodium chloride in $M/1$ solutions.

A similar effect was observed when the transfusions were made with $M/2$ solutions; the onset of rigidity occurred immediately after infusion and persisted for a few days and there was also the same difference in the degree of stiffness between the animals with destroyed cord and those with cords intact.

In the above mentioned two experiments of von Fürth, one with calcium chloride and one with calcium nitrate, ten cubic centimeters of a ten per cent. solution were injected through the aorta of frogs whose cord was destroyed. A ten per cent. solution of calcium chloride is nearly equal to the $M/2$ solutions which we have, among others, employed. Von Fürth thus summarizes his observations: no rigor over night—"Ueber Nacht keine Starre." On account of the divergence of our results with those of von Fürth we shall give an abbreviated protocol of one set of such experiments.

EXPERIMENT 23.—Calcium chloride infusions in the aorta of frogs. Three medium-sized frogs. Frog 1, cord intact. Frog 2, cord destroyed. Frog 3, control. Aorta connected with burette; sinus opened; all three frogs had 30 c.c. 0.6 per cent. sodium chloride solution ran through to wash out the blood. Frogs 1 and 2 had then a transfusion with 12 c.c. calcium chloride in $M/2$ solution.

3.00 p. m. Finished transfusion. Frog 1 stiff, Frog 2 moderately stiff, and Frog 3 flaccid.

4.30 p. m. No relaxation in Frogs 1 and 2; control still soft.

Next day at 12.00 m. and 6.00 p. m., Frog 1 is very stiff, Frog 2 stiff, but less than Frog 1; Frog 3, control, is moderately stiff.

There was no doubt as to the early and persistent rigor of Frog 2—comparable with von Fürth's experiment—although the rigidity was somewhat less than in the frog with intact cord. Could the difference between our experiments and that of von Fürth be due to the difference of the character of frogs? Von Fürth experimented on *Rana temporaria* and we used *Rana esculenta*. It could not be due to the previous washing out with a sodium chloride solution, as we had the same results without such a previous washing. We must again point out that no matter how thorough and extensive von Fürth's experiments otherwise were, on the particular point in question there were only two experiments and they were entirely insufficient to controvert our results which were, moreover, in complete harmony with the results which we obtained in mammals. This agreement was not disturbed by the fact that in frogs even after infusion of magnesium sulphate there was no relaxation of the primary stiffness, since the dose which we employed in frogs, considering the small size of these animals, was in proportion much greater than that employed in mammals.

The fact that in the frogs with intact cord the rigor is stronger than in frogs with destroyed cord has its explanation in that the muscle tissue alone is stimulated in the latter by the concentrated salt solutions, while, when the cord is intact, the simultaneous stimulation of the nervous mechanism increases the motor response of the muscles, which in turn leads to a stronger rigor. The relation of the intensity of the rigor to the stimulating effect of the salts upon the cord reminds one of the strong rigor which follows strychnine poisoning.

In the frogs which received infusions with $M/10$ solutions of the salt the rigor developed late in all animals, and it was difficult to decide whether there was any acceleration or delay in its onset in case of one or the other of the salts.

However, the experiments with transfusions of $M/10$ solutions, as well as experiments in which the animals received in their lymph sacs comparatively large doses of $M/1$ solutions of the salts (about four cubic centimeters for each frog), brought out distinct results

when the posterior half of the body of the frog not skinned was submersed in a sodium chloride bath, kept at a temperature of about 38° C. This method was first successfully employed by Langendorff and Gerlach(41), and later by Nagel(18). Of course, the rigor which sets in here quite early is a heat rigor. But here again the development of the rigor in the calcium animals was far ahead of that of any of the other frogs, which received either one of the magnesium salts or sodium chloride, or were simply control animals. The lower extremities of the calcium frogs pass more or less rapidly through a stage of flexion and abduction into a terminal stage of extreme extension mostly combined with abduction. In the magnesium animals the rigor appears only a little later than in the control animal and the rigid legs remain mostly in a flexed state.

These observations demonstrate that heat rigor also is greatly accelerated by the presence of calcium in the muscles of the animals. The delaying effect of magnesium is not safely established in this line of experimentation.

The experiments on frogs have confirmed the fact that calcium hastens the onset of rigor mortis, and favors also the development of heat rigor. They have also shown that concentrated solutions of calcium and magnesium salts in bloodless animals call forth an immediate contraction of the muscles which passes then without an intermediate stage into the real rigor mortis. As to the delaying effect of magnesium, the experiments on frogs brought no positive results, the failure being essentially due to the late onset of the normal rigor in the animals.

The experiments reported in the foregoing pages have shown that the relations of calcium and magnesium salts to rigor run in opposite directions, that is, calcium hastens and magnesium retards the onset of rigor. We suggested at the outset that if the contraction theory of rigor is correct, both salts ought to retard the development of rigor, that is, on the assumption that both salts exercise inhibitory effects. Can our results be considered as contributing towards a decision between the two theories, namely as being against the contraction theory and therefore in favor of the chemical theory of rigor, and can we assume that the acceleration and retardation of the rigor in our cases have their last cause in the facts established

by von Fürth that calcium salts hasten the clotting of myogen plasma and magnesium salts do not hasten it? It sounds plausible. But there is again some discrepancy between certain points in these facts and certain details in our observations. According to von Fürth(42) magnesium nitrate hastens the coagulation of the muscle plasma, while in our experiments magnesium nitrate has retarded the onset of rigor longer than any of the magnesium salts. Besides, the argument that if the contraction theory be correct, the effects of calcium and magnesium salts ought to be similar, is based, as stated above, on the assumption of the similarity of both salts with regard to their inhibitory effects. Very recent experiments, however, have demonstrated to us that the foundation of this assumption is, to say the least, insecure.

The safer course, therefore, would be not to enter for the present into a discussion of the availability of the facts revealed in this paper in support of one or the other of the theories of rigor.

SUMMARY.

Calcium salts hasten and magnesium salts retard the development of rigor mortis, that is, when these salts are administered subcutaneously or intravenously.

When injected intra-arterially, concentrated solutions of both kinds of salts cause nearly an immediate onset of a strong stiffness of the muscles which is apparently a contraction, brought on by a stimulation caused by these salts and due to osmosis. This contraction, if strong, passes over without a relaxation into a real rigor. This form of rigor may be classed as work-rigor (*Arbeitsstarre*).

In animals, at least in frogs, with intact cords, the early contraction and the following rigor are stronger than in animals with destroyed cord.

If *M/8* solutions—nearly equimolecular to “physiological” solutions of sodium chloride—are used, even when injected intra-arterially, calcium salts hasten and magnesium salts retard the onset of rigor.

The hastening and retardation in this case as well as in the cases of subcutaneous and intravenous injections, are ion effects and essentially due to the cations, calcium and magnesium.

In the rigor hastened by calcium the effects of the extensor muscles mostly prevail; in the rigor following magnesium injection, on the other hand, either the flexor muscles prevail or the muscles become stiff in the original position of the animal at death.

There seems to be no difference in the degree of stiffness in the final rigor, only the onset and development of the rigor is hastened in the case of the one salt and retarded in the other.

Calcium hastens also the development of heat rigor. No positive facts were obtained with regard to the effect of magnesium upon heat rigor.

Calcium also hastens and magnesium retards the onset of rigor in the left ventricle of the heart. No definite data were gathered with regard to the effects of these salts upon the right ventricle.

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TRANSPLANTATION IN MASS OF THE KIDNEYS.

By ALEXIS CARREL.

(From the Rockefeller Institute for Medical Research, New York.)

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I. DEFINITION.

This operation consists of extirpating from a first animal both kidneys, their vessels and the corresponding segments of the aorta and vena cava, their nerves and nervous ganglia, their ureters and the corresponding part of the bladder (Plate XI); of placing this anatomical specimen into the abdominal cavity of a second animal whose normal kidneys have been previously resected and the aorta and vena cava cut transversely (Plate XII); and of suturing the vascular segments between the ends of the aorta and vena cava, and of grafting the flap of bladder onto the bladder of the host (Plate XIII).

II. INTRODUCTION.

The purpose of the transplantation in mass of the kidneys is to reconstruct, as safely and perfectly as possible, the urinary system, when it has been suppressed by a double nephrectomy, and to study the functions of the new kidneys. It would be important to know whether kidneys extirpated from an animal and transplanted on another animal after a suspension of life of some duration, can resume efficiently their functions. No therapeutic value can be expected from the graft of kidneys unless the secretion of the new organs should be practically normal. In order to ascertain whether or not transplanted kidneys functionate in a normal way, the crucial test is certainly the grafting on one animal, having undergone previously a double nephrectomy, of both kidneys extirpated from another animal. The efficiency of the transplanted organs would be absolutely demonstrated if the host lived in good health and secreted normal urine.

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The physiology of transplanted kidneys is not known, the main purpose of the experimenters having been until now to reestablish the circulation through the organ, and not to study its functions. When gangrene did not occur and the kidney secreted a little urine, the operation was considered a success. As the normal kidneys of the host were not resected, it was not possible to appreciate the degree of usefulness of the transplanted organ. All these experiments were made by a method that may be called simple transplantation. It consists of dissecting the kidney and cutting its vessels, extirpating and transplanting it onto some other part of the body of the same or another animal and suturing the renal vessels to other vessels, such as the jugular vein and common carotid artery, or iliac vessels, or even renal vessels.

In 1902 the first attempt of transplanting a kidney was made by Ullmann.² He removed a dog's kidney and transplanted it into the neck, the renal artery being united to the carotid artery and the renal vein to the external jugular vein by means of Payr's prostheses. The end of the ureter was sutured to the skin and fluid flowed from the ureter, but no analysis of it seems to have been made. Three months after this communication Ullmann³ reported having transplanted the kidney of one dog into another, and the kidney of a dog into a goat. On macroscopical examination of the kidney transplanted into the neck several necrotic areas were seen. No analysis of the urine was published.

The same year, I performed⁴ on dogs several transplantations of the kidneys, at the University of Lyons. The carotid and jugular of a dog were dissected and prepared for anastomosis. The right kidney, having been extirpated, with its vessels and its ureter, was put into the cervical wound. End to end anastomoses by continuous sutures of the renal artery to the carotid, and of the renal vein to the jugular, were performed. The end of the ureter was united to a small opening of the skin a little above the sternum. After reestablishment of the circulation a clear fluid began flowing from the ureter. Septic complications occurred in every case, and permanent results were not observed. No analysis of the urine was made. In 1902 Decastello⁵ also reported experiments on the transplantation of the kidneys. He extirpated the kidney of a large dog and transplanted a kidney from another dog into its place, uniting the vessels by means of prostheses. The animal lived forty hours, during which time 1,200 cubic centimeters of urine, containing a great deal of albumin and many casts, were secreted. In 1903 Carl Beck⁶ of Chicago performed a transplantation of the kidney by using Murphy's method of anastomosing blood-vessels.

² Ullmann, *Wien. klin. Woch.*, 1902, xv, 281.

³ Ullmann, *ibid.*, 707.

⁴ Carrel, *Lyon méd.*, 1902, xcvi, 859.

⁵ v. Decastello, *Wien. klin. Woch.*, 1902, xv, 317.

⁶ Beck, verbal communication.

In 1905 Floresco¹ performed the transplantation of the kidney into the cervical and the inguinal regions, and in every case gangrene occurred. Then he grafted the kidney in the lumbar region; a kidney was extirpated from a dog and transplanted on another dog which had undergone the resection of one kidney, the renal vessels being united to the renal vessels of the host by continuous suturing and the ureter to the skin or to the ureter of the host. These experiments gave few facts about the functions of the kidneys. In one case a sample of fluid flowing from the ureter five days after the operation was examined and presented the characters of urea. But on the tenth day the organ was found necrosed. Floresco transplanted the kidney five times into the lumbar region. In three cases necroses occurred. In two cases the animal is reported to have lived in good health. Definitive results were not published. No analysis of urine secreted by the transplanted kidney was made.

In 1905 Guthrie and I² examined the functions of a transplanted kidney. The operation was performed in the Physiological Department of the University of Chicago. The kidney of a small dog was transplanted into the neck, the renal artery being sutured to the carotid artery, the renal vein to the external jugular vein and the ureter to the oesophagus. When examined three days later, the kidney was somewhat larger and its hue darker than normal. The pulsations of the renal artery were strong. The secretion of urine by the transplanted kidney was about five times more rapid than by the normal one. The intravenous injection of normal saline solution caused no change in the rate of secretion in the normal kidney, but markedly increased the rate of secretion in the transplanted organ. The constituents of the urines were similar, but the chlorides appeared more abundant in the urine from the transplanted kidney, while the organic sulphate, pigments and urea were more abundant in the urine from the normal organ.

This experiment showed that the secretion of a transplanted kidney need not be very different from the normal. However, the kidney was in an unfavorable condition. The denervation alone could not be responsible for the exaggeration of the circulation of the organ. In the neck or in the inguinal region the blood pressure is different from the pressure to which the kidney is used. Besides, its vessels, the renal vein especially, are exposed to many causes of compression and disturbance. Therefore, we gave up the idea of transplanting the kidney in a region other than the lumbar region, as Floresco did.

In 1907 Stich³ published the results of a transplantation of the kidney in the lower abdominal region. This experiment was performed at the surgical clinic of Garre, in Breslau. The kidney was transplanted into the iliac region, the renal vessels being anastomosed, end to end, to the iliac vessels, and the end of the ureter being grafted into the bladder. The normal kidneys of the animal were not removed, and consequently, the usefulness of the transplanted organ could not be determined. The circulation remained excellent and the macroscopical and microscopical examinations showed that the organ was practically normal. The iliac region is certainly much better than the cervical region, but

¹ Floresco, *Jour. de Physiol. et de Path. generale*, 1905, vii, 27, 47.

² Carrel and Guthrie, *Science*, 1905, xxii, 473; *Comp. rend. de la Societ  de biologie*, 1905, ii, 669.

³ Stich, *Archiv f. klin. Chirurgie*, 1907, lxxxiii, 494.

it is probable that perfect functional results can be obtained more easily by putting the kidneys in their normal location in the lumbar region.

In 1906 I made with Guthrie¹⁰ a few transplantations of the kidney into the lumbar region. The renal artery and vein were implanted on the wall of the aorta and vena cava by the patching method. By this method occurrence of gangrene is almost impossible, for the wall of the renal vessels is respected and the suture is made between the edges of the arterial and venous patches and the openings of the aorta and vena cava of the host. The circulation was excellent. Nevertheless there were almost always some small changes in the direction, or the situation, and in the length of the vessels, or some twisting of the vein around the artery, or a little sclerosis of the connective tissue which produced a slight obstruction of the venous circulation, chronic congestion, and after a few weeks, marked lesions of the kidney. Such an organ is not proper for the study of the functions of a transplanted kidney, for its secretion is abnormal. Its lesions and troubles of function are not due to the fact of its having been transplanted, but merely of its having been transplanted with an imperfect technique.

For studying the functions of transplanted kidneys, the transplantation in mass appeared to be the ideal method. It permits an almost perfect reconstruction of the urinary apparatus, and prevents the occurrence of gangrene and, in a large measure, of secondary lesions of the parenchyma, since the renal vessels themselves are not disturbed and the ureters not severed. It may preserve a part of the nervous system of the organs, and places the organs in a condition as near as possible to normal.

The first transplantation in mass of the kidneys was performed in 1906 by Guthrie¹¹ and myself in the Physiological Laboratory of the University of Chicago.

Both kidneys and the upper part of the ureters of a dog, together with their vessels, nerves, nervous ganglia, the surrounding connective tissue, the suprarenal glands, the peritoneum and the corresponding segments of the aorta and vena cava were removed. The mass was then placed in a vessel of isotonic sodium chloride solution. The aorta and vena cava of a bitch were cut a little

¹⁰ Carrel and Guthrie, *Jour. of Amer. Med. Assoc.*, 1906, xlvii, 1648.

¹¹ Carrel and Guthrie, *Science*, 1906, xxiii, 394; *Comp. rend. de la Société de biologie*, 1906, i, 465.

above the mouth of the ovarian vessels. The kidneys of the first animal were then removed from the salt solution and put into the abdominal cavity of the second, and the segments of the aorta and vena cava were interposed, by biterminal transplantation, between the cut ends of the aorta and vena cava. The circulation was reestablished, after having been interrupted for one hour and a half. The kidneys immediately became red and turgid, as after a simple transplantation, but about half an hour later the state of the circulation became normal. Clear urine flowed abundantly from the transplanted ureters, which were united to the normal ones.

Both normal kidneys were then removed. Two hours after the operation the animal walked about her cage. In the afternoon she drank and urinated copiously. The following day and subsequently, up to the eighth day, her diet consisted largely of meat, which she took hungrily. In general her condition was normal. During this period the urine remained clear, showing no evidence of blood. The total amount appeared to be somewhat increased. In the seventh and eighth days several samples were collected and analyzed, the results of which showed a slight variation in composition, but entirely within normal limits. The only abnormal constituent detected was coagulable proteid, the largest amount present in any of the samples being less than 0.25 per cent.

On the ninth day she vomited. A diagnosis of stenosis of the bowels by adhesions was made. On the tenth day the urine was analyzed and its composition was practically the same as before. As the animal was vomiting almost continuously, she was etherized and a laparotomy performed. We found localized peritonitis on the right side of the abdomen, with kinking of several loops of intestines, the mass being very strongly bound down by adhesions. The circulation of both kidneys was found to be perfect. There was an enormous hydronephrosis on one side.

Afterwards a few other transplantations on dogs and cats were performed without obtaining better results. The animals died rapidly from intestinal, peritoneal or ureteral complications.

In 1907 at the Rockefeller Institute, I modified and improved the technique in order to suppress, as much as possible, the occurrence of complications. A few operations were made on dogs and on cats and it appeared soon that, in spite of the difficulties due to the smallness of the vessels, cats are better adapted from an anatomical standpoint for this operation than dogs. When the details of the operation had been worked out fourteen experiments were performed from February to October, 1907. Progressively the technique was improved and the cause of the complications which occurred ascertained, and as far as possible suppressed. The actual technique, however, must not be considered as a definitive one. It will be modified in many respects, as greater experience in performing it is obtained.

III. TECHNIQUE.

The transplantation in mass of the kidneys can be described as consisting of four stages: (a) preparation of the kidneys; (b) perfusion and extirpation of the kidneys; (c) preparation of the host; and (d) graft of the kidneys into the abdomen of the host.

It is evident that all resources of modern surgery must be used to prevent infection and shock after such an operation, which necessitates a large transverse incision of the abdominal wall, the evisceration of the intestines and the spleen, a double nephrectomy, the stopping of the aortic and caval circulations, the section of the aorta and vena cava, and the opening of the bladder. The animal is handled and operated on with the same rigid asepsis and care used for a human patient in a well-equipped hospital.

The order of the succession of the different stages of the operation is variable. We shall describe the simplest form, when the four stages are united in two: preparation and extirpation of the kidneys; preparation of the host and graft of the kidneys.

A. Preparation and Extirpation of the Kidneys.

A first animal is etherized. After shaving and sterilization of the abdominal and lumbar regions, the antero-lateral wall is cut transversely at the level of the umbilicus to about one half or three quarters of the circumference of the animal. The intestines are eviscerated and put on the left side of the body. Thus both kidneys and the lumbar region are largely exposed.

1. *Dissection of the Kidneys.*—The peritoneum is cut all around the kidneys in a rectangular shape, the short sides being along the external edge of the kidneys, the long sides uniting the ends of the organs and being perpendicular to the vena cava. Care must be taken not to wound the ureters and the vena cava. Through the peritoneum it is easy to see the point of implantation of the renal veins. At about one centimeter above the mouth of the right renal vein the vena cava is dissected and isolated from the lower end of the right suprarenal gland. On the left side of the vessel, just above the left renal vein, is often the mouth of the left suprarenal vein. This vein is ligated and cut, if the left suprarenal gland is not to be transplanted with the kidneys. Afterwards, the vena

cava is dissected on a point located at two or three centimeters below the mouth of the left renal vein. The left genital vein is ligated and cut. Then the aorta is dissected at two points located one and one half centimeters above and below the renal arteries. The kidneys are detached from the lumbar region by cutting the loose connective tissue which is interposed between them and the muscular plane. The posterior part of the aorta and vena cava is dissected. The posterior collateral branches of these vessels are ligated and cut.

The ureters are dissected from the lower edge of the peritoneal flap to the bladder. Their vessels must be carefully respected. Afterwards the part of the bladder on which the ureters are implanted is dissected. The musculo-peritoneal layer of the bladder is cut by a circular incision located at about one centimeter from the mouth of the ureters. Then the mucous membrane is easily seen and cut, in such a way, with the scissors, that the mucous flap is larger than the muscular one. The ureters and the flap of bladder are still fixed by the rectum and the uterus. The uterus is cut, and the meso-colon severed as far as the cæcum. The small intestine is cut and through the solution of continuity the ureters and the fragment of bladder are removed. Great care is taken to prevent infection by the section of the intestines.

If the left suprarenal must be transplanted, its upper vessels are ligated and cut. The last connective tissue adhesions are severed. The anatomical specimen is yet adherent to the animal by the aorta and vena cava.

2. *Stopping of the Circulation, Perfusion and Extirpation of the Kidneys.*—The aorta is clamped below the diaphragm. A glass cannula is introduced through the lower part of the abdominal aorta and connected with an irrigator containing Locke's solution at the temperature of the laboratory, which was from 26° to 37° C. The vena cava is cut one centimeter above and two or three centimeters below the renal veins. Then the aorta is washed and the kidneys are perfused with Locke's solution. In the first experiments they were washed until their surface became yellowish white and the fluid flowing from the vena cava was perfectly clear. In the last experiments the perfusion was much less complete. It was stopped

as soon as the blood in the vena cava appeared to be greatly diluted, and when there was still a good deal of blood in the kidneys.

Next the aorta is cut one centimeter or one and a half centimeters above and about two centimeters below the renal arteries. The anatomical specimen is removed (Plate XI) and put into a jar of Locke's solution at the temperature of the laboratory.

B. Preparation of the Host, Double Nephrectomy and Graft of the New Kidneys.

A second animal of the same size or a little larger than the first one is etherized. The abdomen and the lumbar region are clipped, shaved and carefully sterilized. The abdominal wall is transversely cut at the level of the umbilicus from one lumbar region to the other. The intestine and the spleen are eviscerated and wrapped in a towel or in a greased silk pad, and put outside on the left side of the body. They are protected there by a small wool blanket, in order to prevent cold and the consequent shock. The aortico-renal region is then largely exposed. When the bladder is too distended by urine, it is put outside of the abdominal cavity and covered with greased pads.

1. *Extirpation of the Kidneys* (Plate XII).—The lumbar peritoneum is cut longitudinally on the middle line, its edges dissected at the level of the renal veins. The pedicles of the kidneys are dissected and a ligature is put on each structure, artery, vein and ureter. Afterwards through the longitudinal peritoneal incision, both kidneys are extirpated in such a manner that the constitution of the renal region is not disturbed at all. Both suprarenal glands are respected, their lower vessels being sometimes ligated. The left genital vein is ligated.

2. *Preparation of the Vessels*.—The vena cava and the aorta are prepared for anastomosis. The vena cava is dissected at the point of implantation of the renal and the left suprarenal veins. One or two lumbar collaterals, and sometimes the right genital vein are ligated. The temporary hæmostasis is secured by *serre fines* especially modified for this purpose. A *serre fine* is put on the vena cava four centimeters below the renal veins, and another one as high as possible, about two centimeters above the right renal.

vein. Then the region on which the renal veins are implanted is resected (Plate XII). Through the cut ends of the vessels a curved glass cannula is introduced, and the blood is washed out with Locke's solution. A little vaseline is put into the lumen and on the external part of the vein. The aorta is generally dissected just below the renal arteries; the first two lumbar collaterals are ligated, a *serre fine* is put on the wall of the vessel just below the renal arteries, and another three centimeters lower. It is better, but involves greater risk, to dissect the aorta above the renal arteries, in order to place the *serre fine* higher and to make the anastomosis a few millimeters below the implantation of these vessels. The relations of the thoracic duct and of the aorta are so close at that point that the duct is exposed to injury while the aorta is being dissected. It is much safer to put the higher *serre fine* below the renal arteries. Generally the aorta is cut two centimeters below the renals, or sometimes a very short segment, with the implantation of the first two lumbar collaterals, is resected. The aorta being very elastic there is immediately a gap of two or three centimeters between the cut ends of the vessel (Plate XII). The external sheath is resected for a short distance. The blood is washed out from the lumen of the vessel with Locke's solution. The lumbar region is carefully washed with Locke's solution and dried with gauze in order to remove all traces of blood. Then the vessels are washed again, and covered with a thin layer of vaseline. Vaseline is also put on the surrounding anatomical structures.

3. *Transplantation of the Kidneys.*—The anatomical specimen is removed from its jar and put into the abdominal cavity. Each kidney is introduced through the incision of the lumbar peritoneum into the corresponding renal region. The new kidneys, being about the same size as the extirpated ones, fill exactly their place. The aortic and caval segments are interposed between the cut ends of the aorta and vena cava. The ends of the vascular segments are washed, if necessary, and greased with vaseline. Afterwards the small region of the vessels is circumscribed with black silk towels, for it is necessary to protect the threads from all contact with blood or with plasmase of the tissues.

4. *Anastomoses of the Vessels and Reestablishment of the Circu-*

lation.—The ends of the aorta are united to the ends of the aortic segment by continuous suturing with straight needles, No. 16, and silk thread boiled in vaseline. Care is taken to express the vaseline from the lumen of the vessel before completing the suture. Afterwards the anastomoses of the ends of the venous segments to the ends of the vena cava are made. The wall of the vein is very thin, and it is necessary to apply intima to intima while suturing, that is, to make a slight eversion of the wall. It is the safest way to prevent inclusion of the external sheath into the line of sutures.

After completion of the anastomoses dry gauze is applied on the anastomoses and the *serre fines* are removed first from the vena cava and secondly from the aorta. For about two minutes a slight compression is made in the region of the anastomosis. Afterwards the gauze is removed and, if there is still a little leakage, one or two complementary stitches are added. It is necessary that no blood at all flow from the line of sutures. The hæmostasis must be absolute.

As soon as the circulation is reëstablished through the aorta, the kidneys begin to be injected with blood, and generally after a few minutes, they assume their normal appearance. The duration of the interruption of the circulation is about one hour. Gauze with Locke's solution is put on the vessels.

5. *Graft of the Flap of Bladder on the Bladder of the Host, and End of the Operation*.—The ureters are extended on the free surface of the peritoneum along the right side of the rectum and along the right uterine horn. The bladder is isolated by pads from the peritoneal cavity. On the middle line of its posterior face a longitudinal incision is made. The flap of bladder is grafted into that opening by muco-mucous, and musculo-muscular continuous sutures. Then the bladder is put back into the abdominal cavity.

The arterial and venous anastomoses are examined again, and if there is slight leakage, it is stopped. If a little blood flows from some small vessels of the connective tissue a ligature is applied, even if the hæmorrhage is exceedingly small. The vaseline and the blood which may be present in the connective tissue around the vessels are washed out with warm Locke's solution.

The lumbar region is closed by suture of the longitudinal peritoneal incision. Thus both kidneys are fixed in their normal location. The intestines and the spleen are put back into the peritoneal cavity. If the intestines are a little cold about one hundred grams of warm Locke's solution are injected into the peritoneal cavity.

The peritoneum and the abdominal muscles are closed by two planes of continuous silk sutures. The skin is sutured with catgut. The dressing consists of talcum powder, gauze, cotton, bandage and a linen shirt.

After the operation the animal is put for two hours into a cage heated to about 30° C. and then into an ordinary cage with screen floor or saw dust. No special care is taken. The animal is given meat and milk one day after the operation. The dressing is removed after about six days. When the urine is to be examined, the animal is put into a metabolism cage with fine screen floor. Only incomplete analyses of urine have been made by using the ordinary clinical methods. In spite of their lack of accuracy they are sufficient to prove that the kidneys functionate. It is not intended, in this article, to analyse minutely the changes in the secretion of transplanted kidney, but merely to show that after transplantation they can resume their function efficiently. This is demonstrated as much by the general condition of the animal as by the physical and chemical characters of the urine secreted by the new kidneys.

IV. EXPERIMENTS.

Fourteen experiments have been performed.¹² Two animals whose ureteral anastomoses were defective died soon after the operation. No excretion of urine was observed from the bladder, because it flowed into the retroperitoneal spaces. Three other animals, operated on under unfavorable condition died almost immediately of shock. These five experiments will not be reported. Thus nine cases only will be described.

Experiment 1. February 25, 1907.

Extirpation of the Kidneys.—Middle-sized male cat. Etherization and semi-

¹² Several of these operations were made with the aid of Mr. R. D. McClure of Johns Hopkins University, whom I wish to thank for his assistance.

circular transversal laparotomy. Evisceration. Dissection and isolation from the surrounding structures of both kidneys, the ~~left~~ suprarenal gland, their vessels and the corresponding segments of the aorta and vena cava, their nervous system, and the upper part of the ureters, which are cut about 6 centimeters below the hilus. Opening of the lower part of the abdominal aorta. Animal killed by hæmorrhage and opening of the diaphragm. Section of the vena cava 1.5 centimeters above and 3 centimeters below the mouth of the renal veins. Introduction of a cannula through the abdominal aorta. Washing of the aorta and perfusion of the kidneys with very hot (by accident) Locke's solution, until their color became yellowish-white and the fluid flowed perfectly clear from the vena cava. Then section of the aorta about 1.5 centimeters above and 1.5 centimeters below the mouth of the renal arteries. Anatomical specimen removed and put into Locke's solution at the temperature of the laboratory (30° C.).

Transplantation of the Kidneys.—Male, young, black cat, good health. Etherization. Semi-circular transversal laparotomy. Evisceration of the intestine on the left side of the body. Longitudinal incision of the lumbar peritoneum in the middle line, through which both kidneys are dissected and extirpated. Dissection of the vena cava at the level of the renal veins. Ligation of two posterior collateral branches. Dissection of the aorta, which is isolated from the thoracic duct. Ligation of both first lumbar collateral branches. Temporary hæmostasis of a segment of aorta by two *serre fines* put on the vessel just above and 4 centimeters below the renal arteries. Section of the aorta a little above the mouth of the first lumbar branches. Resection of a segment of aorta about 5 millimeters long. Temporary hæmostasis of the vena cava by two *serre fines* placed just above the right renal vein and the right spermatic vein. Resection of the point of implantation of the renal veins. Washing of the ends of the vessels with Locke's solution and greasing with vaseline.

The anatomical specimen is then removed from the jar and put into the abdominal cavity, each kidney being placed under the lumbar peritoneum in its normal location. Interposition of the aortic and caval segments between the cut ends of the aorta and vena cava. Anastomosis of the vessels by suture. No expression of the vaseline from the vessels before completion of the suture. The *serre fines* are taken out and the blood flows through the aorta and vena cava. Slight leakage from the venous anastomoses, which stops by compression. No leakage of the arterial anastomoses.

Very slow reëstablishment of the circulation through the kidneys. Nevertheless, after a few minutes, the right kidney assumes a rosy color, without blue or white spots, while the left kidney remains paler. The lower end of the left kidney is white. Small incision made at this point. Slight hæmorrhage of red blood mixed with vaseline. Progressive improvement of the circulation of the left kidney. After thirty minutes its color is rosy. Circulation almost normal.

Invagination of the end of the transplanted ureters into the upper end of the ureters of the host, and fixation of the invagination by three stitches. Closing of the lumbar peritoneal incision. Reintegration of the intestines into the abdominal cavity. Closing of the abdominal wall by three planes of catgut sutures. Gauze and cotton dressing, linen shirt.

February 25, 4 p. m. Cat in good condition. A little depressed.

February 26. Cat in good condition, walks about its cage, drinks water, but does not eat. Femoral pulse normal. A little urine.

February 27. Cat in good condition, drinks water and milk, walks, does not eat meat.—9 a. m. Urinates abundantly.—4 p. m. Urinates again. Yellowish urine, acid reaction, albumin present.

February 28. Good condition. Normal femoral pulse. Animal drinks milk, urinates abundantly, walks about the room. Clear, pale, yellow, acid urine. Albumin.

March 1. Animal in very good condition. Urinates abundantly. Clear, yellowish urine. Less albumin.

March 2. Same condition.

March 3. Animal ill, does not drink milk. Coughs, discharge through the nose. Urinates.

March 4. Animal very ill, coughs, diarrhoea, discharge through the nose. Urinates abundantly.

March 5. Same condition. Animal emaciated. Abundant urine. Drinks water.

March 6. Animal in better condition. Diminution of the discharge through the nose and of the diarrhoea. Drinks milk. Walks about its cage and the room.

March 7. Much better condition. No more discharge. A little diarrhoea. Less abundant urine. Animal hungry and drinks milk, eats fish and raw meat.

March 8. Animal drinks milk and eats raw meat. Urinates. Walks about the room.

March 9. Animal ill, refuses to eat. Urinates as usual, stays in cage.

March 10. Animal very ill. Not able to jump from its cage to the floor. No vomiting, no dyspnoea, no shaking. Urinates. Pressure on the abdominal wall is painful. In one point dressing is wet.—11 a. m. Etherization. Section of the dressing. Several loops of intestines in the gauze, the abdominal wound being almost completely disunited: premature resorption of the catgut. The intestinal loops are partially protected by the omentum which is adherent to the lower edge of the abdominal wound. Comparatively little inflammation of the peritoneum. However, four intestinal loops are adherent to the gauze dressing and very much inflamed. Adhesions are detached and intestine washed. Direct examination of the kidneys: normal peritoneal covering, normal location, marked enlargement, almost normal consistency. They look like normal hydronephrotic kidneys. Washing of the abdominal cavity with warm water. Closing of the abdominal wound by silk suture. Shock. Afterwards respiration and pulse become progressively almost normal.—4 p. m. Animal able to walk.

March 11. Died in the morning.

Autopsy.—General peritonitis.

Macroscopical Examination.—Both kidneys in normal location, covered with transparent peritoneum. Normal hue, increased size. Normal consistency.

The specimen is not dissected, but preserved as demonstration specimen. Incision on the external edge of the left kidney. Urine under pressure flows from the incision. The surface of section is a little congested. No infarction. Dilatation of the pelvis and calices. It is a typical hydronephrotic kidney. Right kidney not opened, but a knife is introduced deeply into the renal substance, and urine escapes along the blade. Same hydronephrotic condition on the right side. Opening of the vena cava. Anastomoses perfect. No dissection of the aorta. No dissection of the ureters. Specimen preserved in formalin.

Microscopical Examination.—Fragment of the left kidney in Zenker's fluid stained in hematoxylin and eosin. Glomeruli well preserved. In some of them slight exudate between capillaries and the capsule. Secreting tubules slightly dilated. Epithelium in good condition; brush border well defined, nuclei normal. Excretory tubules very much dilated. Around some of them slight small-cell infiltration. A little dilatation of the blood-vessels. Appearance of an ordinary hydronephrotic kidney.

Experiment 2.—March 14, 1907.

Extirpation of the Kidneys.—Small pregnant cat etherized and killed by aortic hæmorrhage. Dissection and isolation in one mass of the kidneys, their vessels, their nerves and the upper part of the ureters. Left suprarenal gland not extirpated. By an intra-aortic injection of Locke's solution both kidneys are washed out very thoroughly, until all blood is expelled and their color becomes pure yellowish-white. Anatomical specimen extirpated and put in Locke's solution at the temperature of the laboratory (30° C.).

Transplantation of the Kidneys.—Large, gray, young, male cat. Etherization. Semi-circular transversal laparotomy. Evisceration. Extirpation of both normal kidneys. Dissection of the aorta and vena cava. Ligature of the lower suprarenal vein and section. Ligation of the first lumbar collateral branches of the aorta. Temporary hæmostasis of the aorta by tapes fixed by *serre fines*, and of the vena cava by *serre fines* put directly on the wall of the vessel. Resection of a narrow segment of aorta in which the first lumbar arteries are implanted. Resection of a long segment of vena cava where are implanted the inferior suprarenal vein and both renal veins.

The anatomical specimen is then put into the abdominal cavity, the kidneys being in their normal location and the vascular segments interposed between the cut ends of the aorta and vena cava. Arterial and venous anastomoses by the ordinary method. Reestablishment of the circulation through the vena cava. Leakage at one point of the lower anastomosis; hæmorrhage controlled by one supplementary stitch.

Reestablishment of the circulation through the aorta. Hæmorrhage of the upper anastomosis, which is controlled by two additional stitches. Slow reestablishment of the circulation through the kidneys. Appearance of both kidneys normal twenty minutes after the reestablishment of the arterial circulation.

Anastomoses of the ureters to the upper portion of the ureters of the host by invagination. Suture of the lumbar peritoneum. Reintegration of the intestines in the abdominal cavity. Closing of the abdominal wound by two planes of silk suture and one plane of catgut suture. Gauze and cotton dressing. Shirt.

March 15. Animal in good condition, drinks milk and eats raw meat. Large amount of clear urine. In the afternoon several fits of abdominal pain.

March 16, 9 a. m. Animal less well, refuses to eat, drinks a great deal of water and milk. Since yesterday at 6 p. m. 130 c.c. of urine. Urinates again at 10.30 a. m. yellow, clear, acid urine. A great many spermatozoa, no red blood corpuscles, a little albumin. In the afternoon, fits of abdominal pain, vomited once, no feces. In the evening animal very ill.

March 17. Animal died in the morning.

Autopsy.—A little reddish fluid in the peritoneal cavity. A loop of small intestine is found dilated and of dark color: volvulus. Both kidneys are normal in size, color and consistency. Arterial and venous anastomoses perfect.

Experiment 3.—June 13, 1907.

Extirpation of the Kidneys.—Middle-sized male cat. 10.38 a. m. Etherization. Kidneys exposed and ~~dissected~~ the same as before. Dissection of the aorta and vena cava at two points about 1.5 centimeters above and 2 centimeters below the mouth of the renal vessels. Section of the ureters about 6 centimeters below the hilus. The animal is killed by chloroform at 11.15 a. m. Section of the vena cava. Perfusion of both kidneys through the aorta with Locke's solution at the temperature of the laboratory (30° C.). Complete washing. The fluid from the vena cava is clear, and the kidneys are yellowish-white. Section of the aorta 1 centimeter above and 2 centimeters below the renal arteries. Then the kidneys, the left suprarenal gland, the upper part of the ureters with their vessels and their nerves are removed and put in Locke's solution.

Transplantation of the Kidneys.—Large, yellow, male cat. Etherization. Semi-circular transversal laparotomy. Evisceration of the intestines and the spleen and extirpation of both kidneys. Ligature of the lower suprarenal vein, of two venous collaterals of the vena cava, and the first pair of aortic lumbar collaterals. Dissection of the aorta and vena cava and temporary hæmostasis with *serre fines*. Transverse section of both vessels. The anatomical specimen is then put in the abdominal cavity, the new kidneys being in the same location as the kidneys of the host. Anastomosis of the aortic and caval segments. Re-establishment of the venous and arterial circulation at 12.20 p. m. No leakage of the anastomoses. Immediate reëstablishment of the circulation through both kidneys. No blue or white spots. Normal color and consistency. No apparent vasodilation.

Anastomosis of the ureters by invagination. Suture of the lumbar peritoneum. The intestines and the spleen are replaced into the peritoneal cavity. A few cubic centimeters of Locke's solution are let into the peritoneum. Closing of the abdominal wound by two planes of silk sutures and one plane of catgut. Gauze and cotton dressing. Shirt. Animal is put in a metabolism cage.

June 13, 4 p. m. Animal in satisfactory condition, lays down, does not walk, drinks water.

June 14, 9 a. m. Animal in good condition, walks about his cage, and drinks milk. In the jar 250 c.c. of a mixture of urine, vomitus and a little milk.—9.30 a. m. Animal urinates, clear, slightly rosy urine, quantity 42 c.c., reaction acid, density 1.021, a great many blood corpuscles, a few spermatozoa, no casts.

June 15, 9 a. m. Animal in good condition, drinks milk. Urine 110 c.c. mixed with feces.—3.45 p. m. Clear, rosy urine, with many blood corpuscles.—4.35 p. m. Animal urinates again. Urine 38 c.c., clear, slightly rosy, acid reaction, density 1.018, urea 2.5 per 100 c.c., albumin less than 0.50 g. per 1000 c.c. Many red blood corpuscles, a few spermatozoa, no casts.

June 16, 9 a. m. Vomits a little milk. In the jar 205 c.c. urine and milk.

June 17. Animal in good condition, walks about the room, drinks water, but refuses milk and meat. Does not vomit. Urine mixed with feces.

June 18. Urine 125 c.c., yellowish, acid, density 1.016, urea 2.8 per 100 c.c., traces of albumin. Animal refuses to eat.

June 19. Animal grows emaciated, drinks a great deal of water. Urine 164 c.c., pale yellowish, clear, density 1.013, very little albumin, urea 2.3 per 100 c.c. urinates again 35 c.c. at 1 p. m.

June 20. During the night the animal has escaped from its cage, and urinated on the floor. He is emaciated and refuses to eat.—10.30 a. m. Urinates again, urine 35 c.c., clear, density 1.013 with traces of albumin.

June 21, 7.30 a. m. Urine 115 c.c., alkaline reaction. Density 1.012, urea 2.6 per 100 c.c., no albumin.

June 22, 7.15 a. m. Urine 167 c.c., density 1.017, no albumin. Animal is much emaciated, and does not eat.

June 23. Urine 110 g. Animal is very weak, nevertheless he can walk about the room. The dressing is removed. Wound completely healed. Animal very much emaciated, kidneys a little enlarged. Does not vomit.—11 a. m. Transfusion of blood by Crile's method through an anastomosis of the carotid artery of another cat to the external jugular vein. Blood is transfused until the second animal dies from hemorrhage.

The animal is now in better condition. Pulse much stronger. The mucous membrane of the mouth and the skin of the feet are red. A few minutes after the operation clonic convulsions of the jaws and the limbs. Afterwards tonic convulsions. Tetanus-like appearance.—5 p. m. Died.

Autopsy.—Macroscopical Examination.

Opening of the abdomen a few minutes after death. Excellent healing of the abdominal wound. No adhesions of the intestines. In the gastric region long and narrow strand compressing the duodenum just below the pylorus, without, however, producing complete occlusion. Both kidneys are increased in size, their color, their location and their consistency are almost normal. Section of the right kidney: dilatation of the pelvis and calices, cortex and medulla apparently normal, although a little congested. Capsule normal. Upper part of the ureter greatly dilated. Incision of the right ureter: stenosis of the invaginated part; below this point the ureter normal. The left ureter too is very much dilated and sinuous above the point of anastomosis.

Bladder filled with 35 c.c. yellow, clear urine, density 1.020, urea 2.9, no albumin.

Vessels of the kidneys normal. Aorta and vena cava dissected and incised. No thrombosis or stenosis, perfect anastomoses. No sclerosis of the perivascular connective tissue.

Microscopical Examination.—Piece of the right kidney fixed in Zenker's fluid, stained in hematoxylin and eosin. Glomeruli normal, secretory tubules slightly dilated, epithelial cells in good condition. Excretory tubules somewhat dilated. Very few light casts. No interstitial infiltration. At a few points a few mononuclear leucocytes around the tubules.

Experiment 4.—June 19, 1907.

Preparation of the Host.—Young, white and black male cat in very good health. Etherization. Semi-circular transversal laparotomy. Evisceration, dissection and extirpation of both normal kidneys, ligation of the lower suprarenal vein, of two collaterals of the vena cava and of the first lumbar pair. Temporary hæmostasis. Section of the aorta about 2 centimeters below the renal arteries. Resection of the venous segment on which are implanted the renal veins. Washing and greasing of the vascular ends. Then gauze compresses with Locke's solution are put on the operative field and protected with a wool blanket.

Extirpation of the Kidneys.—Middle-aged, pregnant cat. Etherization. Opening of the abdomen by the ordinary method. Veins extremely dilated. When the anatomical specimen, both kidneys, and left suprarenal gland are almost completely isolated, the ureters are dissected as far as the bladder. Resection of a flap of the vesical wall around their mouths. Then section of the uterus, mesocolon and the small intestine near the cæcum. The ureters and the flap of the bladder are removed from the lower part of the abdomen and placed temporarily below the left kidney. Perfusion of both kidneys with Locke's solution at the temperature of the laboratory (37° C.). The anatomical specimen is removed and placed immediately in the abdomen of the host.

Transplantation of the Kidneys.—Each kidney is placed under the peritoneum in its normal location. Anastomosis of the vessels and reestablishment of the circulation. No leakage. It is noticed that the lower end of the venous segment has been twisted and that it produces a very marked stenosis just above the lower anastomosis. It does not interfere with the circulation of the kidney, the color of which almost immediately becomes normal. The circulation has been interrupted for 42 minutes only. Immediate secretion of clear fluid.

The ureters are placed in the abdominal cavity on the right side of the rectum. Longitudinal opening of the bladder on its posterior face and on the middle line, and graft of the transplanted flap of bladder by muco-mucous and musculo-muscular sutures. Closing of the lumbar peritoneum. Through the lower part of this line of suture a small opening is reserved to allow the ureters to pass from the retro-peritoneal space into the peritoneal cavity. Both ureters are twisted around each other, but, as there is no tension, it probably does not interfere with the flow of urine.

The intestines are put back into the abdominal cavity. Suture of the abdominal wall. Dressing. Shirt.

June 20. Animal in good condition. Urine a little bloody. Does not eat.

June 21. Animal in excellent condition, urinates abundantly, eats a great deal of raw meat.

June 22. Animal apparently in very good health, climbs on roof.

June 23. Dressing is removed, wound completely healed. Animal eats meat and drinks a great deal of milk, urinates abundantly.

June 24. Animal looks well, but refuses to eat.

June 25. Animal in good condition, does not eat. Does not vomit. No feces. Clear, yellow urine, urea 2.2 per cent. A little albumin, less than 0.25 g. per 1000 c.c.

June 26. Animal in good condition, walks, climbs on a wall more than six feet high, but refuses to eat and to drink milk. Then a direct examination of the abdominal cavity is decided upon.

10 a. m. Etherization. Longitudinal laparotomy on the middle line. No intestinal adhesions to the abdominal wall. A few adhesions between the duodenum, the anterior face of the right kidney and the inferior face of the liver. They are loose and easily detached. The kidneys are covered by normal peritoneum and apparently in excellent condition. Size, color and consistency normal. The surface of both kidneys is rosy, there is no apparent vasodilatation. No sclerosis of the connective tissue between the kidneys. The renal veins and the transplanted segment of vena cava appear to be absolutely normal. Normal pulsations of the aorta and the renal arteries.

Then closing of the abdominal wound by four planes of sutures.

One hour after the operation, the animal walks and seems in good condition.

June 27, 8 a. m. Animal found dead in its cage. Body is still warm.

Autopsy.—9.15 a. m.

Macroscopical Examination.—Peritoneum normal, no fluid, no adhesions. Bladder filled with urine.

Both kidneys are in their normal location, covered with sound peritoneum, and normal in color, size, consistency and connections with surrounding structures. From a point corresponding to the lower end of the incision of the lumbar peritoneum, both ureters adherent to one another, penetrate into the peritoneal cavity and go down toward the bladder along the right side of the rectum. Excellent union of the transplanted flap of bladder to the bladder of the host (Plate XIII).

Incision of the peritoneum and dissection of the kidneys. Their appearance is normal. They are surrounded by their covering of connective tissue, which does not present any sclerosis. Dissection and incision of the aorta and vena cava. Anastomoses healed without any deposit of fibrin. Vena cava is obliterated 1 centimeter above the lower anastomosis at the point of the twisting of the transplanted venous segment. This point is much below the mouth of the renal veins and the obliteration did not interfere with the venous circulation of the kidneys. Both kidneys are opened. Capsules normal. No hydronephrosis. In section they have the appearance of normal kidneys. A piece of the right kidney is fixed in Zenker's fluid.

Lungs, heart, liver, spleen apparently normal. No examination of the brain and medulla.

Microscopical Examination.—Glomeruli normal. No exudate in the glomerular space. Epithelial cells of the tubules generally well-preserved, brush border very apparent, regularly disposed and adherent to the basement membrane. In some places vacuolization of the protoplasm around the nucleus. Exudate in the lumen of the tubules. Epithelial cells of the excretory tubules normal. A few typical hyalin casts. No infiltration whatever of the interstitial tissue.

Experiment 5.—June 28, 1907. 7.30 a. m.

Preparation of the Kidneys of the First Animal.—Middle-sized female cat in good health. Etherization. Exposition and isolation of both kidneys, which are abnormally pale, the left suprarenal, the ureters and the corresponding part of the bladder by the ordinary method. The aorta and vena cava are dissected but not cut. The organs are covered with compresses of Locke's solution.

Preparation of the Host.—Strong, slender, young, black cat. Etherization. Exposition and extirpation of both kidneys by the ordinary method. Dissection of the aorta and vena cava, and temporary hæmostasis. Section of the aorta 2.5 centimeters below the mouth of the renal arteries, resection of the segment of vena cava on which the renal veins are implanted, washing and greasing of the vascular ends.

Extirpation of the Kidneys from the First Animal.—Washing of the kidneys by the ordinary method. Section of the vessels. The anatomical specimen is removed and placed in the abdominal cavity of the second animal. The first animal is killed by opening the diaphragm and clamping the heart.

Transplantation of the Kidneys.—The kidneys are put in their normal location and the vascular segments interposed between the ends of the aorta and vena cava. Anastomoses. Because the incision of the abdominal wall is too short, and consequently the operative field narrow and deep, the anastomoses are difficult and a dissecting forceps is frequently used in the handling of the vascular ends. Reestablishment of the circulation 45 minutes after clamping the aorta and interrupting the circulation through the kidneys. After a few minutes the kidneys assume their normal appearance; their surface is rosy without white or blue spots. Opening of the bladder and graft of the flap by two planes of sutures.

Suture of the lumbar peritoneum. Through the lower part of the line of suturing an opening is left for the ureters. The intestines are replaced in the abdominal cavity. End of the operation as usual.

June 29. Animal in excellent condition, urinates abundantly.

June 30. Same condition.

July 1. Slight paresis of the posterior limbs.

July 2. Complete paralysis of the posterior limbs and the tail.

July 3. Animal died.

Autopsy.—Peritoneum, kidneys and all abdominal organs apparently normal. Excellent healing of the flap of the bladder. The bladder is distended with urine. Perfect healing of the anastomoses of the vena cava. The upper aortic anastomosis and the renal arteries are in excellent condition. At about 1 centimeter below the mouths of the renal arteries, the aorta is completely obliterated by a clot. This clot is not adherent to the wall of the transplanted segment or to the line of suture. It is fixed to a wound of the intima of the lower end of the aorta at 1 millimeter below the anastomosis. The wound has been caused almost certainly by the handling of the vessel with the dissecting forceps during the performance of the anastomosis.

As the autopsy was performed a long time after death and the temperature was high, the specimens of kidneys are so poorly preserved that no interpretation of the sections is possible.

*Experiment 6.*¹³—July 12, 1907, 8.15 a. m.

Extirpation of the Kidneys.—Black, pregnant cat, mangy and in bad health. Etherization, extirpation and dissection by the ordinary method, both kidneys, left suprarenal gland, ureters and a large flap of bladder. Clamping of the aorta below the diaphragm at 8.40 a. m. Washing of both kidneys with Locke's solution at the temperature of the laboratory. The perfusion is stopped when there is still a good deal of blood in the kidneys and the veins are still filled with bloody fluid. Then the vessels are cut, the specimen removed and put into Locke's solution.

Preparation of the Host and Transplantation of the Kidneys.—White and black, young female cat in excellent health. Bloody discharge from the vagina. Parturition two days before. Etherization difficult. Semi-circular transverse laparotomy. Evisceration of the intestines, spleen and bladder. Longitudinal incision of the lumbar peritoneum, extirpation of both kidneys, dissection of the

¹³This experiment was reported by Mr. R. D. McClure before the Seventh International Zoological Congress.

aorta and vena cava, and temporary hemostasis. Resection of the segment of vena cava where the renal veins are implanted. Section of the aorta 2 centimeters below the renal arteries. The anatomical specimen is put into the abdominal cavity, the kidneys in their normal location, and the vascular segment between the ends of the aorta and vena cava. Reestablishment of the circulation at 9.45 a. m. No leakage. In a few minutes the circulation of both kidneys is apparently normal. The ureters are put along the right side of the rectum through the basis of the right broad ligament. Incision of the bladder and implantation of the flap of bladder by two planes of sutures. Suture of the incision of the lumbar peritoneum. The intestines and the spleen are put back into the peritoneal cavity. Intestines cold, a little shock. Suture of the abdominal wall by the ordinary method. Gauze and cotton dressing. Shirt. Animal in a metabolism cage.



Cat 6 looking at a piece of meat. Photograph taken on the twenty-first day after the operation.

July 12, 2 p. m. Animal a little shocked, lies down in its cage.

July 13. Animal lies down and refuses to eat. From time to time she gets up, turns around in the cage, and cries as though suffering abdominal pain. Does not vomit. After having urinated she is quiet and looks comfortable again. Urine 120 c.c.

July 15. Animal much better. Walks about the cage, no abdominal pain. Eats a great deal of raw meat, drinks milk, urinates abundantly. Bloody discharge from the vagina. No analysis of urine.

July 16. Animal in normal condition, walks, jumps, climbs, eats a great deal of meat, drinks milk, and urinates abundantly.

July 17, 18. Same condition.

July 19. Animal in perfect health, is growing fat. The dressing is removed. Wound completely healed. Both kidneys normal in size and situation.

July 20, 21 and 22. Same condition.

July 23. Animal fat, and in good health. Eats a great deal of meat. Both kidneys are a little increased in size and less movable.

July 24. Same condition.

July 25. Animal is apparently normal, runs about the roof, climbs and jumps on the table, eats a great deal.

ANALYSIS OF URINE: EXPERIMENT 6.

	Quantity.	Color.	Reaction.	Density.	Urea per 100 c.c.	Albumin per 1000 c.c.	
July 13	120 c.c.	Yellowish pale	Acid	1.007	1.2	less than 0.5	
14							Mixed with feces and milk.
15							
16	140 "	Yellowish	Acid	1.015	1.7	0.5	
17	210 "	Yellowish		1.019	from 2.7-4.2	less than 0.25	
18	95 "	Yellow	Acid	1.018		less than 0.5	
19	82 "	Yellow		1.021	4.9	less than 0.25	
20	120 "	Yellow	Acid	1.026	4.1	none	
21	145 "	Yellow	Acid	1.020	4.4	none	
22	95 "	Yellow		1.022		none	
23	164 "	Yellow		1.029	5.1	none	
24							Mixed with feces.
25	170 "	Yellow		1.035		traces	
26	60 "	Yellow	Acid	1.030		0.75	
27	175 "						Mixed with feces or milk.
28	185 "						
29	255 "	Yellowish		1.019			
30	165 "	Yellowish pale		1.013	1.8	1.25	
31	215 "					1.2	
Aug. 1	160 "						
2	165 "						
3							
4							
5							
6							
7	from						
8	120-160 c.c.						
9							
10						A great deal of albumin.	
11							
12							

I am much indebted for this observation to Dr. Levene and Dr. Auer who, during August, had the kindness to analyze the urine of this animal and to examine her clinically.

July 26, 27, 28, and 29. Same condition.

July 30. Animal is apparently in excellent health. Nevertheless, both kidneys are enlarged and increased progressively in size and are completely fixed and adherent to the lumbar region. They are no longer movable in the abdominal cavity as normal kidneys are.

July 31 and August 1. Same condition.

August 2. A photograph is taken while the animal is eating. (See figure.)

August 3. Animal is apparently in good health, but the kidneys are enlarged and the urine contains more albumin.

From August 3 to August 10 the animal was in excellent condition, eating and acting as a normal cat. On August 11 she began to vomit and in a few hours became very ill. A great deal of albumin in the urine. Died on August 12.

Autopsy.—The body was opened by Mr. McClure. The peritoneum, the intestines, the lungs and the heart were found normal. The kidneys and the vessels were not examined, but the body was put in formalin, and the autopsy completed on October 4.

Macroscopical Examination.—Kidneys in their normal location and greatly enlarged. Normal cicatrization of the flap of the bladder. Both kidneys are strongly adherent to the posterior abdominal wall and to each other. They are united by a growth developed under the peritoneum and passing as a bridge over the aorta and vena cava. This growth is developed outside of the capsule of the left kidney. It is adherent to and interposes itself between the renal vessels, the aorta and vena cava. The vena cava is compressed against the internal face of the right kidney. The right renal vein is also compressed between the tumor and the kidney, while the left renal vein is too much extended. This growth is composed of hard, white, apparently fibrous tissue, well-defined on its anterior side and more diffuse on its posterior side. It is intimately adherent to the transplanted suprarenal gland. Anastomoses excellent. Opening of the kidneys. No hydronephrosis. Congestion. Dilatation of the stellate veins.

Microscopical Examination.—Cadaveric changes of the epithelial cells are so marked that an interpretation of the epithelial lesions is not possible. Glomeruli well preserved, dilatation of the capillary loops which fill almost completely the capsules. Infiltration by plasma cells of the interstitial tissue. This lesion is more marked in the cortex than in the medulla. Focal disposition. Very marked dilatation of the blood-vessels. Appearance of acute interstitial nephritis. Section of the growth shows organized blood clot.

Experiment 7.—July 17, 1907.

Extirpation of the Kidneys.—Pregnant female cat. Etherization. Preparation by the ordinary method of both kidneys, left suprarenal gland, vessels, ureters and flap of bladder. Incomplete perfusion of the kidney as in the previous experiment. Extirpation of the anatomical specimen, which is put into Locke's solution at the temperature of the laboratory (22.2° C.).

Transplantation of the Kidneys.—Young, black, pregnant cat in excellent health. Abdomen considerably enlarged; near the end of pregnancy. Semi-circular transversal laparotomy. Evisceration of the intestines, the spleen, the bladder filled with urine, and the uterus, which is distended by several foetuses. Ligation of the left ovarian vein. Longitudinal incision of the lumbar peritoneum

in the middle line and extirpation of both kidneys. Dissection of the aorta and vena cava, and temporary hæmostasis with *serre fines*. The anatomical specimen is placed in the abdominal cavity by the ordinary method. Reestablishment of the circulation 60 minutes after the interruption. No leakage. Excellent and quick reestablishment of the circulation through the kidneys which assume almost immediately a normal color. Incision of the bladder and graft of the flap of bladder by the ordinary method. After completion of the suture, it is observed that the ureters are twisted around each other. However, the transplanted ureters being very long there is no tension and it will not interfere, probably, with the flow of urine. The intestines, the spleen and the uterus are put back into the abdominal cavity, which is closed by the ordinary method. Gauze and cotton dressing. Shirt.

July 18. Animal lies down, urinates abundantly and looks in good condition.

July 19. Parturition normal. No eclampsia. Urinates and is in good condition.

July 20. Animal apparently normal, walks, drinks milk and eats meat. Bloody discharge from the vagina.

July 21, 22, 23, and 24. Almost the same condition.

July 25, 26. Animal less well, urinates and eats a little meat. High temperature.

July 27, 28. Animal refuses to eat, and looks very ill. However, she urinates abundantly. Bloody discharge from the vagina.

July 30. Animal died.

Autopsy.

Macroscopical Examination.—Large abscess of the pelvis, located on the left side, between the pelvis and the rectum, extending as far as the sub-peritoneal space, and opening to the skin near the anus, on the left side. Big and soft uterus. The left uterine horn is increased in size. On section reddish fluid and a part of a placenta is found.

No peritonitis. Intestines normal. Both kidneys surrounded by loose connective tissue, normal in location, color and consistency. Anastomoses and vessels normal. Slight congestion of the pyramids. No dilatation of the ureters. Excellent healing of the bladder.

Microscopical Examination.—Glomeruli well preserved. No coagulated fluid between the capsules and the capillary loops. It is difficult to appreciate exactly the lesions of the epithelium of the tubules, for the fixation of the specimen is not good. It seems well enough preserved. Slight leucocytic infiltration between certain tubules. Slight dilatation of the blood-vessels.

Experiment 8.—July 19, 1907.

Extirpation of the Kidneys.—Middle-aged pregnant cat. Dissection of the anatomical specimen by the same method as in the preceding experiments. A small quantity of Locke's solution at the temperature of the laboratory (31° C.) is injected into the kidneys. The washing is stopped when the renal vein is still filled with bloody fluid. Anatomical specimen removed and put in Locke's solution at the temperature of the laboratory. Cat killed by hæmorrhage.

Transplantation of the Kidneys.—Long, middle-aged male cat. Semi-circular transversal laparotomy. Evisceration of the intestines and the spleen. Extirpa-

tion of both kidneys. Dissection of the aorta and vena cava, and temporary hæmostasis. Section of the aorta below the renal arteries. Section of the vena cava between the openings of the renal veins. The kidneys are removed from their jar, put into the abdominal cavity and the vessels anastomosed by the ordinary method. Reestablishment of the circulation after 5 minutes after the interruption. No leakage. Excellent circulation through the kidneys, which assume, after a few minutes, their normal appearance. Opening of the bladder and graft of the flap of bladder. Suture of the lumbar peritoneum. It is found that the right ureter goes behind the vena cava and produces a slight degree of compression of this vessel. The intestines are put back into the abdominal cavity. Closing of the abdominal wall by three planes of sutures. Gauze and cotton dressing. Shirt.

July 20. Animal in excellent condition. Urinates abundantly.

July 21, 22. Animal drinks milk.

July 23. Animal apparently in normal condition, walks about the roof. The dressing is removed. Apparent healing of the wound.

July 24. Same condition.

July 25. Animal eats raw meat.

July 26, 27 and 28. Animal in good condition, eats meat.

July 29. Animal is less well. An abscess has developed on the left side below the end of the abdominal suture.

July 30. Animal looks ill. However, he urinates as usual.

July 31. Animal refuses to eat, and looks very ill. The abscess is examined. It is found that it is an extensive and deep abscess of the wall itself, and not merely of the subcutaneous tissue. All the left part of the abdominal wall is involved. Large openings and drainage. In the evening the animal is worse.

August 1. Animal died in the morning.

Autopsy.

Macroscopical Examination.—Large abscess infiltrating the anterior and left parts of the abdominal wall. No peritonitis. Heart, lungs, liver, and spleen normal. Both kidneys are almost normal in size, color, and consistency, and surrounded by a layer of adipose tissue, which has become very abundant below the lower end of the kidneys. Their mobility is normal. Both ureters adherent to one another enter the peritoneal cavity at the level of the lower end of the incision of the peritoneum near the lower end of the left kidney. Perfect union of the transplanted flap to the bladder.

Opening of the bladder. On the posterior wall the flap is found limited by a linear and almost circular scar. The transplanted mucous membrane is normal. The mouth of each ureter presents its ordinary appearance.

Opening of the lumbar peritoneum and dissection of the vena cava. No perivascular sclerotic tissue. The right ureter is twisted around the vena cava. Renal veins almost horizontal, dense connective tissue around the left suprarenal gland. Opening of the vena cava, anastomoses perfectly smooth. A little clot around and in which a short piece of silk thread is found free in the lumen of the vein at 1 centimeter above the upper anastomosis. In a point of the upper anastomosis is a very small red spot where this clot was probably adherent. Dissection of the aorta. The lower anastomosis is almost invisible: at the level of the upper anastomosis, the aorta is adherent to the vena cava by

dense connective tissue. This tissue is removed and, corresponding to the point where it is mostly adherent to the wall of the aorta, a very small opening of the line of suture between two stitches is observed. The aorta is opened: anastomoses smooth and glistening.

Longitudinal section of the kidneys: cortex and pyramids almost normal although a little congested. A small piece is fixed in Zenker's fluid.

Microscopical Examination.—Glomeruli well preserved. Epithelium of the tubules without very marked lesions. Very few casts. Extensive foci of infiltration of the interstitial tissue by plasma cells; subacute interstitial nephritis. Slight dilatation of the blood-vessels.

Experiment 9.—October 14, 1907.

Dissection and Preparation of the Kidneys.—Middle-aged female cat. Etherization. Preparation of the anatomical specimen as usual. The lower suprarenal vein is ligated and the gland is not included in the specimen. Section of the uterus and the intestine. The specimen, of which the circulation is not interrupted, is protected by the omentum and towels.

Preparation of the Host.—Gray and white female cat, living in the laboratory several months; young and in good health. October 1. Urine examined—yellow, clear urine. Density 1.039. Urea 5.9 gr. per 100 c.c. No albumin. Etherization. Opening of the abdomen and evisceration as usual. The intestines are protected by Japanese silk towels sterilized in vaseline. Dissection and extirpation of both kidneys. Ligature of the ovarian veins, of the lower suprarenal vein and of two aortic collaterals.

Extirpation of the Kidneys.—Clamping of the aorta below the diaphragm at 11.25 a. m. Incomplete washing of the kidneys with Locke's solution. Section of the vessels and removal of the specimen.

Transplantation of the Kidneys.—The specimens are placed immediately in the abdominal cavity of the host. After temporary hæmostasis with *serre fines*, the aorta is cut 3 centimeters below the renal arteries. Resection of the region of implantation of the renal veins. Washing and greasing of the vascular ends. Anastomosis of the vessels. Reestablishment of the circulation at 12.15 p. m. No leakage. Excellent circulation through the kidneys and the small vessels of the lower ends of the ureters. Nevertheless, the left kidney remains pale, while the right kidney is rosy and secretes urine. Opening of the bladder and graft of the flap by two planes of stitches. After the completion of this suture, a slight hæmorrhage from the lower arterial anastomosis is noticed and controlled by one stitch.

The appearance of the kidneys is now very much modified. The left kidney is vasodilated, and its vein carries red blood, while the right kidney has become pale and vasoconstricted, and its vein is filled with dark blood.

End of the operation as usual.

October 14, 4 p. m. No shock. Animal walks, drinks water, has urinated 9 c.c. of bloody urine, the blood depositing itself quickly at the bottom of the glass, urine clear on the top. Urea 4.9.—11 p. m. Urinates again, much less blood.

October 15. Animal a little sick, drinks water, walks about the cage.—4 p. m. Since yesterday 25 c.c. urine only, dark yellow, with a little blood. Albumin. Density 1.051.

October 16. Animal in better condition, drinks milk and eats a little meat. Urine 16 c.c.

October 17, 18. Good condition, drinks milk, is given very little meat.

October 18. Same condition.

October 19. Cat in excellent condition. He is given plenty of milk and very little meat.

October 20. Same condition.

October 21. Cat is completely recovered. He is a normal cat, in the same condition as before the operation.

October 22. Diet from now raw liver and milk.

October 23. Dressing is removed; wound completely healed.

October 24. Cat normal. By palpation the kidneys are found small and movable. They are apparently normal.

October 25. A sample of urine is examined. Clear, yellow urine, acid, density 1.030. Urea 5.1. No albumin.

October 26. Cat normal, kidneys normal in size and movable.

October 27. Same condition.

October 28. Cat being normal is allowed to go out from the cage and to run freely through the room.

October 29. Cat is put into another room and spends all day climbing on and jumping off the furniture.

October 30. Normal general condition, kidneys a little enlarged and less movable.

October 31. Cat a little depressed. The kidneys are very much enlarged and fixed to the lumbar wall. Samples of urine are then examined.—1 p. m. Pale yellow, clear urine. Urea 3.6. Marked quantity of albumin.—3 p. m. Heavy precipitate of albumin by nitric acid.—6 p. m. Albumin 6 gram per 1000 c.c.

November 1. Cat is a little depressed, but still in very good general condition. The kidneys are much enlarged. Very large amount of albumin.—10.30 a. m. Etherization. Semi-circular transversal laparotomy, just above the scar. A few adhesions of the omentum to the wall. Peritoneum and intestines normal. Both kidneys appear very much enlarged and covered with sound peritoneum. Their consistency is a little softer than normal. Incision and dissection of the lumbar peritoneum on the middle line. There is a little sclerosis of the sub-peritoneal connective tissue around the anterior side of the vena cava. It may possibly produce a slight degree of compression of the vessel. Nevertheless, the arterial and venous circulation appear to be normal. The connective tissue of the hilus is oedematous, clear fluid flows after incision. The wall of the ureter is oedematous, without congestion. The small vessels are distinctly seen with red blood. The color of both kidneys is rosy and normal. There is no congestion. Incision of the capsule of the right kidney: clear fluid and red blood flow. The tissue of the kidney is incised: it is oedematous and not congested. Abundant hæmorrhage. Suture of the capsule with Lyon's silk and needles, No. 16. Both renal veins of the right kidney are dissected. The circulation is normal. The perivascular connective tissue is not sclerotic but oedematous. During the dissection of the upper vein several blue, round spots are seen on the surface of the organ, and disappear after a few minutes. No suture of the incision of the lumbar peritoneum. Abdominal wound closed as usual.

6.30 p. m. Cat in good condition. Urine 2 c.c. Much less albumin, 2.75.

November 2, 8 a. m. Cat in good condition, but depressed. Walks about cage. Takes milk only, possibly a little meat.—10.30 a. m. Urine 19 c.c. Albumin 0.60.

9 p. m. Urine 52 c.c. Density 1.020. Albumin 1.50. Urea 4.2.

November 3. Same condition. Diet consists only of milk. Cat depressed.

November 4. Better condition. 8 a. m. Urine 61 c.c., dark yellow, density 1.033. No albumin.

November 5. Same condition.

November 6. Albumin again 1.50.

November 7. Cat depressed, walks about its cage.

November 8. Better condition. Eats a little fish.

November 9. Cat in better condition, but very emaciated. Therefore, he is given rare liver and codfish and eats hungrily. Dressing removed. Wound healed. Both kidneys much diminished in size, but still abnormally large.

November 10. General condition improved.

November 11. Both kidneys diminish steadily in size. Cat eats codfish.

November 12. Cat eats meat and fish. From 9 a. m. to 6.30 p. m., urine 48 c.c., clear yellow. Density 1.021. Urea 3.2. Albumin 1 gr.

November 13. Cat in good condition. Albumin less than 1 gr.

November 14. Cat well in the morning. In the evening, looks ill. Discharge from the nose. Refuses to eat.

November 15. Abundant nasal discharge. Cat refuses to eat. Urine, yellow clear. Albumin less than 1 gr.

November 16. Animal weak and emaciated. Very abundant purulent nasal discharge. The quantity of urine during the last twenty-four hours was 78 c.c., yellow. Density 1.031. Albumin 1.80. Urea 4.8. Many red blood corpuscles and granular casts. The size of the kidneys almost normal.

November 17. Animal very emaciated, weak, but still able to jump from his cage and walks about the room. Abundant nasal discharge. Refuses to eat.

November 18. Animal very weak.

November 19. 1 p. m. died. Post-mortem 2.15 p. m. Opening of the abdominal cavity. A loop of jejunum is adherent to the posterior abdominal wall. Sharp flexure without obstruction. Both kidneys normal in location, color, size and consistency. Ureters normal. Perfect healing of the transplanted flap of bladder. In the bladder, yellow clear urine 5 c.c., albumin. Longitudinal incision of the kidneys, which are apparently normal. However the cortex is pale. Medulla normal. Capsule normal. No dilatation of the stellate veins.

The main pathological change is a general and intense calcification of the arterial system. The arteries are as hard and friable as thin-walled glass tubes. These lesions have developed since the transplantation, for the cat was a young animal in excellent health and its abdominal aorta was perfectly normal.

V. RESULTS.

The results of the experiments will be examined from the clinical and anatomical standpoints.

A. Clinical Results.

In every case the reestablishment of the renal functions was observed. These functions were determined by the characters of the urines and the general condition of the animals.

The secretion of urine may begin as soon as the arterial circulation is reestablished. In several cases, clear urine flowed from the ureters while the flap of bladder was being grafted onto the host. More often, no urine was seen flowing from the ureters immediately after completion of the operation. But the secretion always began during the first twenty-four hours. It is difficult to ascertain exactly the amount of urine secreted during the first few hours, because of the vomitus and water which are often mixed with urine. However, Cat 6 did not vomit, and after the first twenty-four hours the jar contained 120 cubic centimeters of urine. On the other hand Cat 1 urinated very little on the first day. Cat 9 urinated only 25 cubic centimeters during the first twenty-four hours; the second day the amount of urine passed was only 16 cubic centimeters, this urine was highly concentrated and contained much urea. These differences in the immediate amount of urinary secretion are due probably to unknown conditions of the vasomotor nerves. It is generally supposed that denervation of the kidney produces the secretion of an abundant and diluted urine. In the simple transplantation of the kidney, when, for instance, an isolated organ is transplanted into the neck, these phenomena were observed. But in the case of transplantation in mass, immediate vasodilatation is not so marked. Sometimes there is vasoconstriction, but oftener the kidneys retain their normal appearance. Exceptionally, vasodilatation alternates with vasoconstriction. In Experiment 9 about ten minutes after the reestablishment of the circulation, the right kidney was rosy, its venous blood red, and some urine flowed from its ureter, while the left kidney was pale, and apparently did not secrete. About thirty minutes afterwards, when the suture of the bladder was completed, both kidneys were examined again. The conditions were now reversed: the right kidney had become pale, and its vein filled with dark blood, while the left kidney was rosy and its vein contained red blood. It seems

that following transplantation the renal ganglia begin to act and that variable conditions of the nervous system may be responsible for the differences in the immediate results observed.

In all the experiments the urinary secretion went on as long as the animal lived. Every cat urinated abundantly every day. But the animals presented sooner or later some complication, which modified in some measure the renal functions. As is to be expected after an operation as complex as the transplantation in mass, various accidents occurred; hydronephrosis, intestinal compression by peritoneal adhesions, volvulus, phlegmon, puerperal infection, compression of the renal veins by organized hæmatoma of the connective tissue, which were the direct or indirect causes of death in these animals. It is well known that several of the complications, especially the compression of the renal veins, produce grave renal lesions of their own. Therefore, the results of our experiments must not be considered as expressing generally the normal condition of transplanted kidneys, but merely of transplanted kidneys when subjected to various complications, that is, of more or less abnormal transplanted kidneys. Actually, it is impossible to know exactly how a normal transplanted kidney would functionate, for we cannot as yet discriminate between the disorders produced by such a common complication as hydronephrosis or compression of the veins and the less defined ones which may be due to lesions produced by the transplantation itself. However, in Experiment 6 and 9, for instance, the functions of the kidneys seem to have been for a certain time almost completely normal.

The color of the urine was yellow, generally or often less dark than the normal urine of the cat. Its reaction was acid. Its quantity for twenty-four hours oscillated between 120 and 160 cubic centimeters. But it might be, exceptionally, 25 and even 15 cubic centimeters, or, in another case, 215 or 255 cubic centimeters for twenty-four hours. In this case there was congestion of the kidneys produced by venous compression. The density was very far from constant; generally it oscillated between 1.018 and 1.030, going sometimes as high as 1.035 and 1.051. In Experiment 6 there was little parallelism between the amount of urine and the

density. Once the kidneys secreted 170 cubic centimeters of urine with a density of 1.035.

In all cases the amount of urea bore a relation to the diet of the animal. In Cat 6, abundantly fed with raw meat, the amount of urea varied from 2.7 to 5.1 grams. Cat 6 passed through his own kidneys, fourteen days before the operation, 5.9 grams of urea for 100 cubic centimeters. Eleven days after the operation, he eliminated through his new kidneys 5.1 grams of urea for 100 cubic centimeters. The difference is explained by the diet which was less abundant after the operation than before.

Among the abnormal constituents of the urine, the presence of albumin only has been looked for. In Experiment 1 albumin was present during the fourteen days of the post-operative life of the animal. These kidneys were abnormal owing to the perfusion with too hot Locke's solution and to a developed hydronephrosis. In the other cases there was little albumin during the first days, ranging from 0.50 to 0.25 gram for 1000 cubic centimeters. This was probably due in part to the blood coming from the suture of the ureter or the bladder. The amount of albumin decreased progressively and disappeared about one week after the operation. In Experiment 6 albumin was again found on the thirteenth day after the operation, and its amount increased progressively to 1.50 grams and beyond. In Experiment 9 there was albumin in the urine one day after the operation. On the eleventh day no albumin at all was present. On the fifteenth and sixteenth days the animal was allowed to run and climb freely. On the seventeenth day albumin was found again in marked quantity and, at the same time, enlargement of the kidneys was distinctly detected by palpation.

The general condition of the animal can be used, in some measure, to indicate the perfection of the urinary elimination. As long as no complications were present, the animals lived as normal cats do, without presenting any symptoms which could be considered as produced by renal insufficiency. When general complications occurred the cats reacted against them in normal ways.

Cat 1 suffered from eventration, due to a premature resorption of the catgut in the abdominal suture. In two other animals sutured with the same catgut, the resorption and eventration

occurred on the fifth or on the seventh day after the operation. We may admit that in Experiment 1 the resorption took place at about the same time. However, in spite of the extrusion of intestine outside of the abdominal cavity in the gauze dressing, the animal lived several days, drank water and milk and ate a little meat, and urinated abundantly. When the reduction of the inflamed intestines into the abdominal cavity was performed, fourteen days after the operation, the animal was still able to overcome the operative shock, and to walk about the room a few hours later.

In Experiment 6 the animal was in apparently normal condition four days after the operation. She walked about the room, played and ate a great deal of raw meat. Her condition remained excellent for several weeks. Twenty days after the operation she was in good health, had glossy hair, was very fat and ate with appetite all kinds of food. She ran about the room, played, jumped and climbed on the desks and tables as a normal cat does (see figure). There was, however, albumin in the urine, and slow and progressive enlargement of the kidneys took place, which showed that she was not in an entirely normal condition. Nevertheless, until the twenty-ninth day after the operation, she seemed to be in excellent health. Then gastro-intestinal symptoms appeared and death occurred on the thirty-first day after the operation. In Experiment 7 the animal operated on was a pregnant cat whose uterus contained several large foetuses. After the operation, she was immediately in good condition. Two days afterwards parturition occurred without any eclamptic fits or any abnormal symptoms. As the animal seemed to recover very easily and began eating meat one day afterwards, she was not observed very carefully. The dressing was removed and she was let alone. When she was examined again, eight days after the operation, she was found less well and feverish. Her condition grew worse and she died thirteen days after the operation. The autopsy showed puerperal infection with retention of a placenta and an enormous abscess of the pelvis.

Experiment 9 was a female cat which lived in the laboratory for several months. She was in excellent condition when she was operated on, and recovered very quickly from the operation. Her life went on just the same as before. The kidneys were movable

and small. She looked in excellent health and lived as a normal cat. On the eighteenth day after the transplantation a direct examination of the kidneys was made to ascertain the cause of the appearance of albumin. The general condition was little affected by the operation and the albumin disappeared on the twenty-first day, but reappeared again a little later. On the thirty-fifth day, the animal was very weak and emaciated. She died on the thirty-sixth day.

We can conclude from these results that the functions of the kidneys reestablish themselves after the transplantation. Since an animal, such, for instance, as Cat 6, can live in an apparently prosperous condition of health fifteen or twenty-five days, and more, after a double nephrectomy, and eliminate each twenty-four hours 120 and 160 cubic centimeters of urine through the new kidneys, it is certain that the functions of the transplanted organs are efficient. Even these functions during a part of the life of animals No. 6 and 9 can be considered as having practically been normal. When complications contingent or inherent to the actual method of transplantation occurred, the functions of the kidneys were modified and became abnormal according to the pathological changes suffered by the organs.

B. *Anatomical Results.*

The Blood Vessels.—The condition of the blood vessels was examined three times by laparotomy on the living animal and in the other cases at the autopsy.

The direction of the vena cava was almost always found normal. Once, however, the interposed segment was too long and bent. In Experiment 9 the position of the veins was modified considerably by an organized hæmatoma which had pushed the vena cava against the right kidney. The right renal vein was compressed and the left one too much extended. This diminished the activity of the venous circulation and produced marked congestion of the kidneys. It is very important that the veins be given their normal situation and direction. On account of the low pressure and the thinness of the wall, they are not able to take care of themselves as arteries do. Every departure from the normal produces a diminution of

the caliber and consequently slight or marked congestion of the transplanted organ.

The relations of the veins with the surrounding structures, arteries and ureters were generally normal. It happened once that by mistake the right ureter was twisted around the lower part of the venous segment. It did not appear to cause any marked disturbance. The vessels were free in loose connective tissue, excepting in Experiment 6 where the vena cava and renal veins were compressed by an organized hæmatoma. When progressive compression of the veins had occurred it was expressed clinically by progressive enlargement of the kidney, and by reappearance of albumin in the urine on the thirteenth day after the operation, while the animal appeared otherwise in perfect health. In another case there was a little sclerosis of the perivascular connective tissue, which produced some retraction of the right kidney toward the middle line. The induration or sclerosis of the connective tissue may be a serious secondary complication. It occurs in the transplantation in mass but more often in simple transplantation, and oftenest in transplantation with implantation of the renal vessels on the aorta and vena cava. The induration has no influence on the arteries, but interferes with the venous circulation. As soon as the vein is no longer able to dilate freely in the loose connective tissue of the hilus, its circulation is slightly hampered and the result is a chronic congestion of the organ. This sclerosis may be brought about also by slight non-suppurative inflammation or perhaps by chemical irritation. It is probable that this condition is often due to a slight infiltration of blood into the connective tissue. Blood has an irritative influence on tissues. In Experiment 8 there were strong adhesions between the aorta and vena cava, at the level of the upper arterial anastomosis. The vessels were connected by a mass of dense connective tissue. The aorta was dissected, and the maximum of adhesion was found to be on the anastomosis itself and on this point there was a small gap between two stitches. The connective tissue was probably produced under the influence of the infiltration of blood through this opening. Many examples of this sclerotising influence of blood have been observed after transplantation of segments of vessels. It is well

known that extravasation of blood in joints, muscles, or in the central nervous system produce hard connective tissue. Thus injection of blood has been utilized by Bier and Schmieden¹⁴ for inducing callous formation in cases of pseudarthrosis. The perivenous sclerosis, which is a dangerous secondary complication in transplantation of organs, can be prevented probably by rigid hæmostasis and asepsis.

The venous anastomoses healed without thrombosis or stenosis. In one case there was an obliteration of the vena cava due to a torsion of the vein. But this was quite independent of the anastomosis. In Experiment 8 the venous anastomosis was normal. Nevertheless, a very small ovoid clot, which developed around a fragment of silk thread, was found free in the lumen of the vein, about one centimeter above the upper anastomosis. On the anastomosis itself by minute examination a little red spot was detected, which might have been the point where this clot was adherent. This is an absolutely exceptional complication.

The aorta and the transplanted aortic segment assumed in every case a normal direction and appearance. The direction of the renal arteries was the reverse of normal. This was due to the fact that the transplanted segment was fixed on the aorta below the implantation of the normal renal arteries. A modification of direction has no harmful influence on the arterial circulation. The thickness of the wall and the high blood pressure allow the arteries to adapt themselves to abnormal situations. Only one complication was observed: in Experiment 5 there was a complete obliteration of the lower part of the venous segment and the lower end of the aorta by a thrombus. This thrombus was dissected and found adherent to a wound of the intima, just below the lower anastomosis. The wound was evidently produced by the dissecting forceps used in this case for handling the vessel.

The anastomoses healed without thrombus or stenosis. The intima of the transplanted segment was smooth and glistening. No deposit of fibrin was observed, and consequently no embolus. In Experiment 1 a fatty embolus was noticed. A few minutes after the reëstablishment of the circulation, the right kidney assumed

¹⁴ Schmieden, *Jour. of Amer. Med. Assoc.*, 1907, xlviii, 395.

a rosy and normal color, while the left kidney remained pale. The lower end especially was almost completely yellowish white. An incision through the capsule was made at this point. A small hæmorrhage of red blood mixed with a good deal of vaseline followed, and stopped after a few moments. However, the circulation of the left kidney improved progressively and after thirty minutes was almost normal. It is probable that the vaseline under the progressive increase of temperature of the kidney became more fluid and flowed through the capillaries. The anatomical examination of the kidneys, fifteen days after the operation, showed no evidence of this embolus. The few accidents described could be almost completely prevented by operating on animals of a larger size.

The Nervous System.—The anatomical conditions of the nervous system of transplanted kidneys are not yet known. The attempt was made to preserve as completely as possible the circulatory apparatus of the nervous ganglia with the hope that they would resume their functions partially. It is not impossible to believe that sympathetic ganglia of which the vessels are respected and the circulation reëstablished immediately after the transplantation do not degenerate completely. It has been shown, especially by the experiments of Morat,¹⁵ that sympathetic nerves have some of their trophic centers in the ganglia. A part of the vasoconstrictor nerves of the tongue, contained in the hypoglossus nerve, have a trophic center in the superior sympathetic ganglion. After section and degeneration of the sympathetic, stimulation of the hypoglossus still produces vasoconstriction of the tongue. After extirpation of this ganglion, stimulation of the hypoglossus becomes negative. After intra-cranial section of the facial nerve on a dog, almost all vasomotor fibres degenerate from six to twenty-six days after the operation. However, the stimulation of the chorda tympani produces a slight vasodilatation of the gland. Morat assumes that in this case the geniculatus ganglion must be considered as a trophic center for these fibers.

Even if it be admitted that nervous ganglia can, in some measure, functionate when severed from the central nervous system, it is

¹⁵ Morat, *Comp. rend. de l'Acad. des sciences*, 1897, cxxiv, 1389.

not certain that transplanted ganglia can recuperate their functions. The experiments of Stewart and Guthrie¹⁶ show that after an acute and complete anæmia of the nervous centers for more than twenty minutes, the reestablishment of their functions is not possible. The functional activity of the ganglia should necessitate also the reestablishment of a practically normal circulation. The experiments of Tuckett¹⁷ have demonstrated that if the vascular supply of the upper sympathetic ganglion is deranged, degeneration sets in forthwith. But, by preserving the connective tissue surrounding the pedicle of the kidneys and not cutting the small collateral branches of the aorta and vena cava, the vascular apparatus of the kidneys can possibly be kept in its integrity. In this condition, the ganglia may resume their functions, if it be physiologically possible.

Ureters and Bladder.—Dilation of the ureters and hydronephrosis took place in the cases where anastomoses of the ureters were performed. In Experiment 3 the upper part of both ureters was very much dilated and the invaginated part stenosed. However, the urine flowed into the bladder satisfactorily, as is shown by the clinical history of the animal. The ureters of a cat are so small that the anastomosis is very difficult, and stenosis or disunion occurs. On small animals it seems proper to give, up completely uretero-ureteral anastomoses.

The results of the graft on the bladder of the host of a fragment of bladder extirpated around the points of implantation of the ureters were excellent. The anatomical specimens showed that there was no distension of the upper part of the ureters or the kidneys. Both ureters, adherent to one another, entered the peritoneal cavity through the lower part of the incision of the lumbar peritoneum and went downward along the right side of the rectum. In one case they were twisted around one another; in another, the right ureter was twisted around the vena cava. In spite of these faults of technique the functional and anatomical results were very satisfactory.

In every case, the union of the flap of the bladder of the host took place. After opening the bladder, the transplanted flap ap-

¹⁶ Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.*, 1906, viii, 289.

¹⁷ Tuckett, *Jour. of Physiol.*, 1905, xxxiii, 77.

peared congested and swollen, or entirely normal with the same color and appearance as the surrounding mucosa from which it was separated by a linear scar. On the surface of the transplanted mucosa both openings of the ureters were distinctly seen, normal in size and appearance.

The Kidneys.—The kidneys were examined three times only on living animals. During the operations performed on Cats 1 and 9, fourteen and eighteen days after transplantation, the kidneys were seen covered with sound peritoneum and as regards their color, situation and general appearance, they looked just like ordinary hydronephrotic or oedematous kidneys. In other cases, the anatomical examination was performed after the autopsy. For this part of the work, I am very much indebted to Dr. Simon Flexner, who had the kindness to look over the specimens and histological sections and to give me the invaluable help of his advice.

Macroscopical Examination.—In the experiments where there was no hydronephrosis or venous compression, the size of the kidneys was normal. Hydronephrotic and congested kidneys had their ordinary appearances. Their location was always normal. They remained at the place where they were put during the operation and maintained by the suture of the lumbar peritoneum. As a rule, they were not as movable as the cat's kidneys are normally. However, in Experiments 6 and 7 for instance, their mobility was practically normal. The kidneys of Cat 6 were strongly united to each other and to the lumbar wall by hard connective tissue. In the cases where no hydronephrosis or congestion took place, the consistency of the organ was normal. The external and internal appearance of the organs presented no special characters. They looked like congested, hydronephrotic, or almost normal kidneys. The kidneys of Cat 6 were very much congested. The capsule was slightly adherent to the parenchyma. The stellate veins were very much dilated and the medulla and cortex much increased in size.

In Experiments 1 and 3 the urine flowed from the organs when opened, the calices and pelves were dilated, and the surface of the parenchyma congested. The kidneys were ordinary hydronephrotic

organs. In the other experiments, the kidneys assumed the appearance of normal or slightly congested organs.

Microscopical Examination.—The specimens were generally fixed in Zenker's fluid and stained in hæmatoxylin and eosin. Some of them were taken from the animal several hours after death. In Experiments 6 and 7, the body was simply opened and put in a jar of formalin, while the pieces for histological examination were cut from the kidneys two months after. These faults of technique explain why in several cases the specimens were so badly hardened and why there were such cadaveric changes, especially in the epithelium of the tubuli contorti that an interpretation of the pathological lesions was difficult. It was found that the kidneys presented some lesions, very slight in some cases and more marked in others. In Experiment 4, for instance, the glomeruli and the epithelia of the tubules were very well preserved. There was no interstitial infiltration, and a few casts only were observed. The lesions of these kidneys were very slight.

The lesions noticed in the other experiments belong to two classes; hydronephrosis and nephritis. Hydronephrotic lesions were observed in Experiments 1 and 3. The excretory tubes were very much dilated. There was, too, some dilatation of the tubules of which the epithelium was flattened. In a few places between the tubes slight interstitial infiltration was present. In Experiment 3 the glomeruli were normal. In Experiment 1 some coagulated fluid was seen between Bowman's capsule and the capillary loops. The changes of the epithelium of the tubules were slight. The cells were regularly disposed inside the basement membrane, and the brush border was distinctly seen. The lumen contained some fluid exudate, but very few casts were observed. All these lesions may be explained by the presence of hydronephrosis.

Inflammatory lesions were present in three cases; very slight in Experiment 7, but more marked in Experiments 6 and 8. The epithelial degeneration was not extensive in Experiment 8. It seemed more marked in the other cases, but the cadaveric changes were so pronounced that no accurate interpretation was possible. The characteristic lesion met with in these three cases was the infiltration of the interstitial tissues between the tubules. The most

marked case was Experiment 8, in which the foci of infiltration were extensive. The infiltration was composed of cells having the characters of the plasma cells described by Councilman¹⁸ in acute interstitial nephritis, so-called.

This subacute interstitial nephritis is not a necessary complication of the transplantation, since it was absent from the first cases. It is due probably to secondary causes, physical or chemical conditions of the fluid used in the perfusion, congestion of the organ, diet, general condition of the animal, etc. Many factors may come into play during and after the transplantation for injuring the kidneys. The interstitial and epithelial lesions are due doubtless to some of these. It was regarded as surprising that comparatively few changes were found in the renal structure considering that the organs had been exposed to the rough handling of the transplantation. The cells of the secretory epithelium of the kidney are extremely delicate and sensitive to the modifications of the circulation, etc. It is well known that temporary ligature of the renal vein produces extensive degeneration of the epithelial cells. The simple suspension of the circulation has a harmful influence on the epithelium. The cadaveric disintegration of the cells of the tubules begins very early. One hour after death, the brush border has almost always disappeared.

In the transplantation, the renal tissue is not only deprived of circulation for one hour at least, but is also subjected to a perfusion with a fluid which exerts probably its own harmful influence. The perfusion of the organs seems necessary for preventing the formation of clots and the occurrence of thrombosis of the vessels or infarcts of the kidneys. The solution employed is the ordinary Locke's solution. It has been chosen because it is a physiologically balanced fluid. Pure sodium chloride solutions have injurious effects on the tissues. Ringer has shown that minute amounts of calcium and potassium salts antagonize the effects of the pure sodium salt. Loeb¹⁹ and his pupils have laid special emphasis on the poisonous effects of pure sodium chloride solution. Fundulus eggs put in a pure sodium chloride solution of the same concentration as sea

¹⁸ Councilman, *Jour. of Exper. Med.*, 1898, iii, 393.

¹⁹ Loeb, *Amer. Jour. of Physiol.*, 1899-1900, iii, 327.

water cannot live, but they can live if a definite proportion of calcium chloride be added. Howell²⁰ and Harvey Cushing²¹ also showed the injurious effects on the heart and muscles of the pure sodium chloride solution. In their experiments on tumors, Flexner and Jobling²² found that the percentage of successful transplantations is much higher when the fragments of tumor have been preserved in Ringer's instead of salt solution. Therefore, in the perfusion or washing of delicate anatomical structures, physiologically balanced solutions must always be used. But even such a solution is harmful if it has not the same osmotic tension as the tissues. It is very probable that the osmotic tension of Locke's solution is not exactly suited to the cat's kidney. Rathery²³ has shown that slight variations of the cryoscopic point of the solution in which a fragment of the kidney is preserved is able to modify in a large measure the morphology of the cells. For the rabbit's kidney, the best solution has a cryoscopic point of -0.78 C. All other solutions are nephrolytic. If, for instance, a solution which freezes at -0.90 C. is used, the cells are found retracted and as having expelled into the lumen of the tubules a great many of their nuclear granulations. In order to prevent the osmotic disturbances that Locke's solution probably produces in some measure on the cat's kidney, it would be necessary to determine accurately the cryoscopic point of the solution isotonic for the cat's kidney, and to use then a balanced solution of this same tension.

Nevertheless, even an iso-osmotic, physiologically balanced solution would not be able to keep the kidney in its normal condition. Salkowski²⁴ has shown that organs kept at body temperature under conditions which prevent bacterial growth undergo self-digestion. The autolysis of liver and kidneys, etc., is due to proteolytic enzymes which are contained in the cells and come into play as soon as the circulation is stopped. Opie²⁵ succeeded in isolating two proteolytic

²⁰ Howell, *Amer. Jour. of Physiol.*, 1898, ii, 57.

²¹ Cushing, *ibid.*, 1901-02, vi, 77.

²² Flexner and Jobling, verbal communication.

²³ Rathery, *Le tube contourné du rein, étude histologique, anatomopathologique expérimentale*, Thèse de Paris, 1905.

²⁴ Salkowski, *Zeit. f. klin. Med.*, 1890, Suppl., xvii, 77.

²⁵ Opie, *Jour. of Exper. Med.*, 1907, ix, 207.

ferments from the leucocytes and the lymphocytes, leucoprotease and lymphoprotease, and discovered that these enzymes are held in check by an antibody present in the serum. He was able also to isolate this antibody and to show that in a relatively small quantity it can neutralize the action of the autolytic ferments.

The researches of Opie explain why a mineral solution is not able to preserve tissues in normal condition, since autolysis soon occurs. To hold in check the activity of the proteolytic ferments set free by the suppression of the circulation, it is necessary to use a fluid containing in some proportion the antibody of the serum. The simplest method would be to use normal serum for perfusing the kidney. Another method consists of cooling immediately the organ to $+1^{\circ}$ C. a temperature at which the enzymotic activity is almost completely suppressed.

In the first experiments, the kidneys were thoroughly perfused with Locke's solution. In the last ones, the perfusion was very incomplete, a great deal of blood being still mixed with Locke's solution. This change was made with a view of leaving in the vessels of the kidney a little normal serum and contained anti-enzymotic bodies. But the amount was probably insufficient to prevent autolysis.

After the circulation has been reëstablished, the kidneys are not, however, in normal condition, and their cells are still exposed to many causes of injury.

The blood pressure of the host may differ from that to which the kidneys were accustomed. Possibly, the serum of the host is injurious, in some cases, to the new organs. Generally, however, the serum of an animal has no cytolytic action on the cells of another animal of the same species. Nevertheless, Ehrlich has shown that isocytolysins exist. Consequently, it may happen that the cells of the transplanted kidneys are injured by the serum of the host, even of the same species. This is probably an exceptional complication.

The isolation of the nervous apparatus of the kidneys from the central nervous system may be the cause, direct or indirect, of anatomical lesions. The denervation appeared as a grave objection to the possible efficiency of the transplanted kidneys. There is no

physiological evidence of the existence of secretory renal nerves. However, denervation of the kidney produces, according to Bindo de Vecchi,²⁶ degenerative lesions of the epithelial cells of the tubules. Marked disorders of the renal functions followed the section of the nerves in the experiments of Krimer, Brachet, Muller and Peipers. The urine, very abundant and diluted, contained albumin and even blood corpuscles. It is, of course, impossible to ascertain whether these changes are due to the section of the hypothetical secretory nerves or merely of the vaso-motor nerves or, perhaps, to other secondary causes. But, even if the denervation alone be able to bring about these results, it need not be considered as especially dangerous. An animal can live in good health after section of the renal nerves. Floresco²⁷ dissected and cut out the nerves of the left kidney of a dog. Fifteen days afterwards, he resected the right kidney. The animal remained in good health. Last year, an experiment still more complete and conclusive was performed by me at the Rockefeller Institute. In the same operation, the right kidney of a bitch was extirpated and the left kidney isolated and left united to the body only by its ureter and vessels which were dissected as closely as possible. Practically all the nerves were severed. Nevertheless, five days after the operation, the amount of urine voided was 130 cubic centimeters. There was no albumin and the animal was in a normal condition. After one month the animal was found secreting from 90 cubic centimeters to 124 cubic centimeters of urine with high specific gravity and without albumin in the twenty-four hours. Eight months after the operation, he was in excellent health. This demonstrates that the denervation of the kidneys is of little importance for the general health of the dog. It is probable, however, that the kidneys, being deprived of the powerful protection of their nervous system, are more sensitive to pathological insults than the normal kidneys are. Had the animal been allowed to live as street dogs do instead of being kept quietly in a cage at an even temperature, with good food and without muscular exertion, perhaps pathological changes would have ensued.

²⁶ de Vecchi, *Arch. di farmacol. sperimentale e scienze affini*, 1906, v, 433, 479.

²⁷ Floresco, *loc. cit.*

CONCLUSION.

Among so many etiological factors, it is impossible to discriminate which are responsible for the complications which took place in our experiments. An attempt to explain the occurrence of nephritis, oedema or calcification of the arterial system, for instance, will not be made, but the technique of the operations will be modified in order to suppress as much as possible the causes which may originate these secondary changes. The purpose of this article was not to analyze minutely the physiological or pathological character of the functions of transplanted kidneys, but merely to ascertain whether these functions are efficiently reestablished.

It is to be concluded that an animal which has undergone a double nephrectomy and the grafting of both kidneys from another animal can secrete almost normal urine with his new organs, and live in good health at least for a few weeks. This demonstrates that it is possible to reestablish efficiently the functions of transplanted kidneys.

EXPLANATION OF PLATES.

PLATE XI.

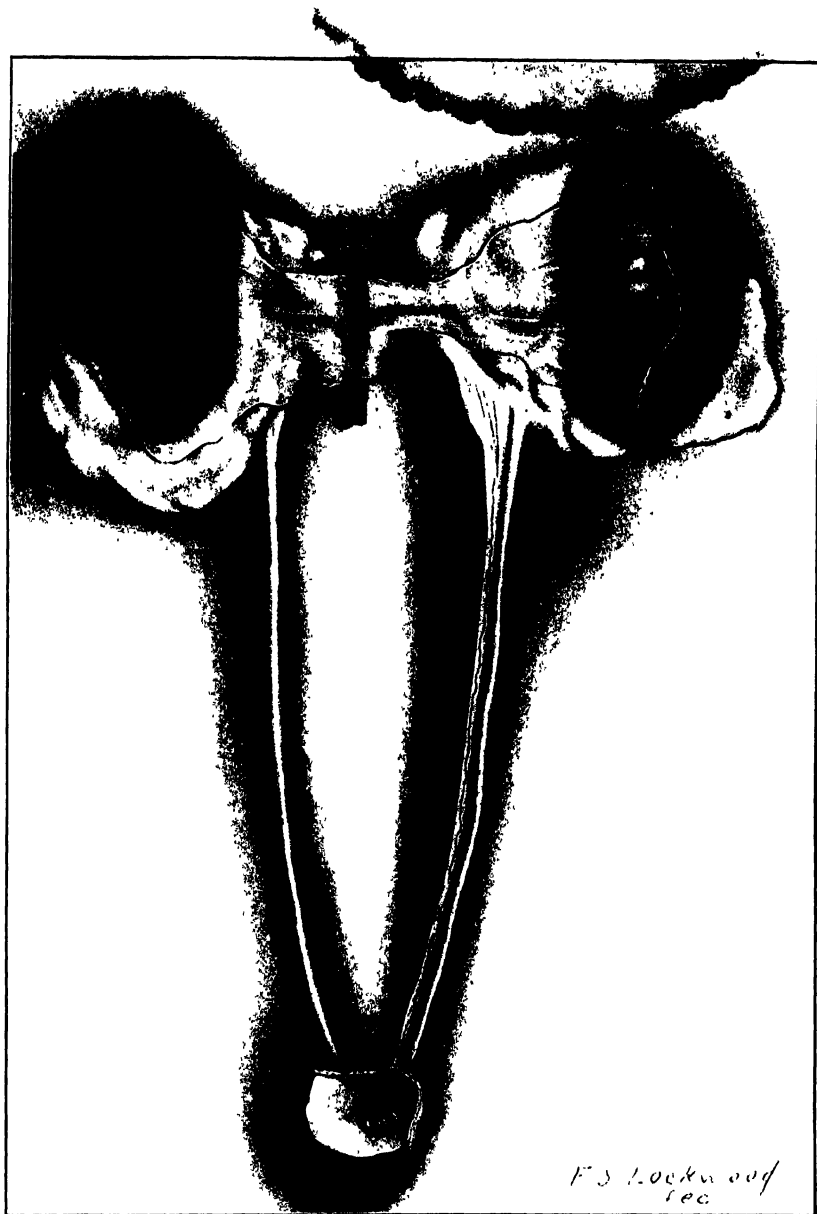
Anatomical specimen extirpated from the first animal and ready for transplantation to the second animal (host).

PLATE XII.

The host ready for the reception of the anatomical specimen of Plate I.

PLATE XIII.

Specimen taken from Cat 7, showing the transplanted kidneys, and cicatrized vascular anastomoses and flap of bladder.







ON THE INHIBITORY INFLUENCE OF EOSIN UPON
SPORULATION.

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Sporulation requires optimum temperature and suitable nutrient media. It is greatly influenced by various physical and chemical agents. Thus, in the case of *Bacillus anthracis*, the most studied in this respect of all spore-bearing organisms, no sporulation takes place at temperatures above 42° C.² or below 14° C.³ Weil⁴ places the lowest temperature at which sporulation takes place at 7° C. The presence of oxygen seems to be essential to the formation of anthrax spores.⁵ Persistence of conditions unfavorable to sporulation through many successive generations of the organism gives rise to asporogenous strains in which virulence is often found greatly reduced or even totally absent.

Phisalix⁶ succeeded in obtaining an asporogenous strain of *B. anthracis* by cultivating it at 42° C. for twelve successive generations. Roux⁷ induced a similar biological alteration by means of a medium containing certain chemicals. Potassium bichromate in the ratio of 1 to 2000, or phenol in from 2 to 6 to 10,000 added to ordinary bouillon stops sporulation completely. Behring⁸ found that various acids, alkalies, salts and certain antiseptics when used in suitable concentrations prevent sporulation. The ant sporulative property of certain dyes was also described by Behring,⁹ who states that safranin in 1 to 30,000, and methylene violet, cyanid, and malachite green in 1 to 200,000 to 1 to 600,000 exert a powerful restraining influence upon the growth and sporulation of *B. anthracis*. After

¹ Received for publication October 1, 1907.

² Phisalix, *Bull. méd.*, 1892, vi, 533.

³ Kitasato, *Zeit. f. Hygiene*, 1890, viii, 198.

⁴ Weil, *Zeit. f. Hygiene*, 1901, xxxvi, 451.

⁵ Schreiber, *Cent. f. Bakt.*, 1896, xx, 353. Weil, *Arch. f. Hygiene*, 1901, xxxix, 205. Klett, *Zeit. f. Hygiene*, 1900, xxx, 420. Jacobitz, *Cent. f. Bakt.*, 1901, xxx, 232. Slupski, *Cent. f. Bakt.*, 1901, xxx, 396.

⁶ Phisalix, *Bull. méd.*, 1892, vi, 533.

⁷ Roux, *Annales de l'Inst. Pasteur*, 1890, iv, 25.

⁸ Behring, *Zeit. f. Hygiene*, 1889, vi, 117.

⁹ *Idem*, 1889, vii, 171.

two months' successive cultivation in the colored agar media, no permanent loss of the sporulating property resulted.

Schreiber¹⁰ states that potassium phosphate of a concentration above 3 per cent. prevents sporulation of *B. anthracis*, *B. subtilis* and *B. tumescens*. Behring,¹¹ Bormans,¹² and Lecleff¹³ found that *B. anthracis* does not form spores in blood serum, while Brieger, Kitasato and Wasserman¹⁴ recorded a few instances in which *B. tetani* failed to sporulate in an aqueous extract of thymus gland.

While abundant work has been done with various spore-bearing aerobes, especially with *B. anthracis*, a similar study with anaerobic organisms has been so far neglected. In a recent study on the antitetanic property of certain dyes, Flexner and Noguchi¹⁵ called attention to the fact that eosin in an adequate strength prevents the sporulation of *B. tetani*, the experimental details of which are given in a later paper by Noguchi.¹⁶

In the present communication I wish to present some of the results of experiments on the restraining influence of eosin upon the sporulation of various microbes. The varieties of bacteria subjected to experiment belonged to the aerobic and to the anaerobic organisms. Of the first, *B. anthracis*, *B. megatherium*, *B. cereus*, *B. mesentericus*, *B. subtilis*, *B. ruminatus*, and *B. anthracoides*, and of the second, *B. tetani*, *B. anthracis symptomaticus*, *B. botulismus*, *B. œdema maligni*, *B. enteritidis sporogenes* and *B. putrificus* were studied.

Eosin "Gelb" having been mixed with agar or bouillon in varying strengths, the inoculations of bacteria were made as usual. Stab and slant solid cultures were made. For the anaerobic bacteria, tissue-bouillon and a deep layer of glucose agar were employed. The agar tubes were incubated in an atmosphere of hydrogen. The results are tabulated.

Table I. indicates that the inhibitory action of eosin is most intense upon *B. cereus* and *B. mesentericus*, and least upon *B. anthracoides*, while *B. subtilis*, *ruminatus*, *anthracis* and *megatherium* occupy intermediary positions. All growth became uncertain when the concentration of eosin reached one per cent.; below this

¹⁰ Schreiber, *Cent. f. Bakt.*, 1896, xx, 353.

¹¹ Behring, *Zeit. f. Hygiene*, 1889, vi, 117.

¹² Bormans, cited in *Baumgarten's Jahresberichte*, 1895, xi, 138.

¹³ Lecleff, *La Cellule*, 1894, x, 349.

¹⁴ Brieger, Kitasato and Wassermann, *Zeit. f. Hygiene*, 1892, xii, 137.

¹⁵ Flexner and Noguchi, *Jour. of Exper. Med.*, 1906, viii, 1.

¹⁶ Noguchi, *Jour. of Exper. Med.*, 1907, ix, 281, 291.

TABLE I.—*Examination after 10 Days.*

Slant Agar with Eosin "Gelb."	<i>B. cereus.</i>	<i>B. mesentericus.</i>	<i>B. subtilis.</i>	<i>B. ruminatus.</i>	<i>B. anthracoides.</i>	<i>B. anthracis.</i>	<i>B. megatherium.</i>
Control (no eosin).	+ all	+ all	+ all	+ all	+ all	+ all	+ all
0.001 per cent.	+ few	+ few	+ many	+	+	+	+
0.01 "	—	—	+ few	+	+	+	+
0.05 "	—	—	—	+?	+	+?	+?
0.1 "	—	—	—	—	+	—	—
0.5 "	—	—	—	—	—	—	—
1 "	—	—	—	—	—	—	—
2 "	—	—	—	—	—	—	—

+ = spore formation.

— = no spore formation.

+? = sporulation doubtful.

strength, multiplication of the bacteria still takes place. These organisms, when cultivated in bouillon containing varying amounts of eosin "Gelb," appear to be even more sensitive to the action of the dye than when grown on a slant agar surface. But when the cultures are allowed to stand for many weeks, sporulation takes place in a medium containing as high concentration of the dye as 0.1 per cent. Concentrations above 0.3 per cent. prevent sporulation, to which effect is added restraint of growth and formation of chains of bacteria through imperfect multiplication. At times, marked degrees of involution occur. In no instances was growth discovered in bouillon containing two per cent. of the eosin.

In deep stab cultures the results were practically identical with those given in the table. Transplantation of the asporogenous bacilli into eosin-free media was associated with immediate return of the spore-bearing power. Many weeks' contact of the sporeless vegetative bacilli with the eosin exerted no enduring effect on the sporogenous property.

The influence of eosin "Gelb" on the sporulation of the anaerobic species has been shown in Table III. Recapitulated, they show that

TABLE II.

Bouillon Culture with Eosin "Gelb."	B. cereus.	B. mesentericus.	B. subtilis.	B. ruminatus.	B. anthracoides.	B. anthracis.	B. megatherium.
Control (no eosin)	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 25 d. +	3 d. + 25 d. +
0.001 per cent.	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 25 d. +	3 d. + 25 d. +
0.003 per cent.	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. - 25 d. +	3 d. - 25 d. +
0.01 per cent.	3 d. - 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. - 25 d. + few	3 d. - 25 d. +
0.03 per cent.	3 d. - 38 d. +	3 d. + 38 d. +	3 d. - 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. - 25 d. + few	3 d. - 25 d. +
0.1 per cent.	3 d. - 38 d. +	3 d. - 38 d. +	3 d. - 38 d. +	3 d. - 38 d. +	3 d. - 38 d. +	3 d. - 25 d. +	3 d. - 25 d. +
0.3 per cent.	3 d. - 38 d. -	3 d. - 38 d. -	3 d. - 38 d. +	3 d. - 38 d. -	3 d. - 38 d. -	3 d. - 25 d. - ?	3 d. - 25 d. -
1 per cent.	No growth	3 d. - 38 d. -	3 d. - 38 d. -	3 d. - 38 d. -	3 d. - 38 d. -	3 d. - 25 d. -	No growth
	No growth	No growth.	No growth	No growth	No growth	No growth	No growth

d = day or days.

+ = spore formation.

- = no spore formation.

TABLE III.—*Observations made after 29 Days on Certain Anaerobes Cultivated Aerobically in the Presence of Tissue*

Bouillon Containing a Small Piece of Rabbit's Fresh Liver.	<i>B. tetani.</i>	<i>B. anthracis</i> symptomaticus.	<i>B. botulinus</i>	<i>B. oedema</i> maligni.	<i>B. enteritidis</i> sporogenes.	<i>B. putrificus.</i>
Percentage eosin "Gelb" in the bouillon						
Control (no eosin)	+	+	+	+	+	+
0.001 per cent.	+	+	+	+	+	+
0.003 per cent.	+	+	+	+	+	+
0.01 per cent.	—	+ ?	+	— ?	—	—
0.03 per cent.	—	—	—	—	—	—
0.1 per cent.	—	—	—	—	—	—
0.3 per cent.	—	—	—	—	—	—
1 per cent.	No growth	No growth	No growth	No growth	No growth	No growth

+ = spore formation.

— = no spore formation.

? = spore formation doubtful.

the eosin in the strength of one per cent. completely inhibits all multiplication of the bacteria. The results are only very slightly different in the case of the cultivation of the anaerobic species freely in the air, in the presence of tissue (Table III.) and in an atmosphere in hydrogen in deep glucose agar. The phenomena of restraint are less pronounced in the latter media.

SUMMARY.

Sporulation of *B. anthracis*, *B. subtilis*, *B. cereus*, *B. ruminatus*, *B. mesentericus*, *B. anthracoides* and *B. megatherium* does not take place in an agar medium containing eosin "Gelb" in a concentration exceeding 0.5 per cent. In a concentration of 0.1 per cent. most of these bacteria fail to produce spores. The greatest sensitiveness is shown by *B. cereus* and *B. mesentericus*. In a bouillon medium sporulation is likewise inhibited by eosin, but after a longer time—seven weeks or more—sporulation still occurs where the concentration of the dye equals one-tenth per cent.

Sporulation of *B. tetani*, *B. anthracis* symptomaticus, *B. botu-*

lismus, *B. oedema maligni*, *B. enteritidis sporogenes*, and *B. putrificus* does not take place in a medium containing eosin "Gelb" in concentrations exceeding 0.03 per cent. With these organisms, no difference was noted in the final effect, depending on the medium employed. No permanent loss of power to produce spores ensues with the bacteria tested even after long sojourn in the eosinized media.

It may be stated that on the whole the inhibitory action of eosin was more pronounced upon the anaerobic than upon the aerobic species of bacteria employed in these experiments.

THE PURGATIVE INEFFICIENCY OF THE SALINE CATHARTICS WHEN INJECTED SUBCUTANEOUSLY OR INTRAVENOUSLY.

A REPLY TO BANCROFT.

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In an interesting article, published a few years ago, J. B. MacCallum stated that "*all those salts which act as purgatives when introduced into the stomach or intestine have the same action when injected subcutaneously or intravenously.*"¹ His conclusion was of interest for the following reasons: in the first place, it was in opposition to the results obtained by most other investigators,² and secondly, it enriched therapeutic measures, for it frequently might be desirable to administer purgatives by another way than the mouth and the small doses employed seemed to permit this.

As a reinvestigation seemed desirable the therapeutically important salts were tested regarding their purgative effect when injected subcutaneously or intravenously. In these experiments the same animals (rabbits) and the same doses which MacCallum had utilized were employed. The salts used were magnesium sulphate, sodium sulphate, sodium phosphate and sodium citrate. The results obtained³ did not confirm the generalization of MacCallum quoted above; moreover, not only did no purgation occur but in some cases a definite constipation seemed to be produced. Tables were given in the published report which illustrated the uselessness of the salts mentioned as purgative agents when injected into the subcutaneous tissue or the bloodstream. The

¹ MacCallum: *Amer. Journ. of Physiol.*, x, p. 102, 1903. Italics not mine.

² For references, see Auer: *Amer. Journ. of Physiol.*, xvii, p. 15, 1906.

³ Auer: *ibid.*, p. 25.

results seemed so clear and convincing that the question could be regarded as settled in favor of the older investigators. Recently, however, Bancroft¹ has come forward with both a critique of my work and a claim that "MacCallum's results have been confirmed in every respect"² by him.

Before entering upon a discussion of Bancroft's paper, I may state at once that he not only utterly fails to explain my results but that his own work, when analyzed, also fails to corroborate MacCallum.

Bancroft's Criticism.

Bancroft gives the following explanations³ for my failure to obtain MacCallum's results:

(1) That I used only the "milder purgatives" employed by MacCallum.

(2) That I kept no adequate controls.

In regard to the first objection it must be pointed out that naturally only those salts were tested regarding which there was a doubt, and those salts were the ones employed in human therapeutics: magnesium sulphate, sodium sulphate, sodium phosphate and sodium citrate. Barium chloride was not used, because no one doubts its purgative effect when injected subcutaneously or intravenously. Moreover, I fail to understand why my use of the saline cathartics in the dosage employed by MacCallum constitutes a reason for my failure to obtain MacCallum's results: a two to sixfold increase in the fecal output.⁴

The second objection is even more ill-founded, and is best answered by a glance at a résumé of some of my published tables.

In the 36 experiments published in the former paper,⁵ 21 showed a *zero output one hour after* the injection; 27 showed not more than *two grams of feces after one hour*; and 25 passed not more than *four grams of feces during five to six hours* after the injections.

¹ Bancroft: *This Journal*, iii, p. 191, 1907.

² *Ibid.*, p. 193.

³ *Ibid.*, p. 210.

⁴ MacCallum: *loc. cit.*, p. 103.

⁵ Auer: *op. cit.*

February 15.	Total feces in 5 hours.	February 16.	Total feces in 6 hours.
Rabbit 1.....	0 grams	Rabbit 5.....	0
Rabbit 2.....	3 grams	Rabbit 6.....	0
Rabbit 3.....	1 gram	Rabbit 7.....	4 grams
Rabbit 4.....	1 gram	Rabbit 8.....	0
Control 1.....	1 gram	15 cc. 25 per cent solution of sodium sulphate subcutaneously.	
Control 2.....	14 grams		
15 cc. ^M / ₈ sodium sulphate subcutaneously.			
March 3.	Total feces in 6 hours.	March 9.	Total feces in 6 hours.
Rabbit 13.....	0	Rabbit 13.....	0
Rabbit 14.....	13 grams	Rabbit 14.....	1 pellet
Rabbit 15.....	1 gram	Rabbit 15.....	0
Rabbit 16.....	2 grams	Rabbit 16.....	4 grams
15 cc. ^M / ₈ sodium phosphate subcutaneously.		2 cc. ^M / ₈ sodium sulphate intravenously.	
March 13.	Total Feces in 4 hours.	March 8.	Total feces in 5 hours.
Rabbit 13.....	0	Rabbit 9.....	0
Rabbit 14.....	0	Rabbit 10.....	0
Rabbit 15.....	0	Rabbit 11.....	0
Rabbit 16.....	7 grams	Rabbit 12.....	0
2 cc. of a 25 per cent solution sodium sulphate intravenously.		2 cc. ^M / ₈ sodium phosphate intravenously.	

These tables show that the output of feces in most experiments was practically zero for four to six hours after the injection of sodium sulphate and sodium phosphate, subcutaneously or intravenously; the doses and concentrations employed were those used by MacCallum, with the exception of the two series where a 25 per cent solution of sodium sulphate was used. Now what could controls show with regard to these experiments? If the controls also passed no feces in the same length of time as the experimental rabbits this could only mean that the injected solutions had no purgative effect; if the controls passed more than the injected rabbits, this again could only mean that the injected solutions constipated the animals. *This fact, that a minimal or zero fecal output of the injected animals during the experimental time required no controls, was early realized, though*

not early enough to avoid considerable work studying the amount of feces passed by normal rabbits when confined in separate cages for some time.¹ Yet Bancroft says that adequate controls would have shown the increase in the amount of feces which I was probably getting.² Even a cursory glance at the published tables should have shown him that his explanation demanded the excretion of practically less than no feces from those "adequate controls" in the majority of experiments. It is therefore clear that Bancroft's "explanation" of my results is unfounded.

Bancroft's Experiments.

Bancroft (p. 193) divides his experiments into two groups:

(1) "Those which simply repeated MacCallum's experiments, small doses being used."

(2) "Those in which the largest possible doses were used, in order to obtain fluid feces."

A consideration of the experiments quoted shows that Bancroft did not repeat MacCallum's work. Bancroft habitually uses 30 cc. of an $\frac{M}{16}$ solution, while MacCallum used only 10 to 15 cc. of an $\frac{M}{8}$ solution;³ the latter calls this amount a "fairly large quantity," while Bancroft calls two to three times that quantity of a *stronger* concentration, a "small dose." Moreover Bancroft's repetition confines itself to the subcutaneous injection of sodium citrate only. No intravenous injections of the ordinary salines, using MacCallum's doses (1 to 2 cc. of an $\frac{M}{8}$ solution), are given in his paper.

Let us now consider what Bancroft's experiments which form the basis of his Table I really show. He used four rabbits of approximately the same size and weight and confined them in separate cages. "In one pair the same rabbit was always the control, in the other pair *one animal was control one day and the experimental animal the next.*"⁴ This means that two rabbits received 30 cc. of an $\frac{M}{16}$ sodium citrate solution subcutaneously

¹ Auer: *loc. cit.*, p. 15. In the course of this paper a few tables showing the hourly output of feces passed by a number of normal rabbits when kept in separate cages will be given.

² Bancroft: *loc. cit.*, p. 210.

³ MacCallum: *loc. cit.*, p. 103.

⁴ Bancroft: *loc. cit.*, p. 193-194. Italics mine.

every other day; one rabbit never received any injection at all; and the fourth rabbit received 30 cc. of the solution every single day. He also changed the diet of the rabbits, which had been fed on hay, grain and vegetables, to only carrots and water. The feces of all the rabbits were then weighed after certain intervals, and Bancroft figures out, using the statistical method, that the injected animals passed during the first three to five hours after the injection, 23 times more feces than the "controls."¹ *He overlooks an important point however in making this calculation. On the same page he mentions that "a period of constipation follows the purgation due to the citrate."*² This in order to explain why the rabbits passed feces only on alternate days. *But these constipated rabbits were used as controls in the experiment.* If Bancroft thought these animals constipated, why did he use them as adequate controls? This error explains his astonishing result. The only control in the series was Rabbit 3 which received no injection whatsoever, and of this rabbit Bancroft³ states that it had "the diarrhoea," though the output to me seems perfectly normal and comparable to many which I have obtained. *It is clearly evident therefore that all of Bancroft's calculations based on Table I are worthless, and they will not be considered further here.*

This table however furnishes some other information; it shows what an extremely modest output of feces Bancroft is willing to consider a purgation. In his Table I the record of 16 injections of sodium citrate into three rabbits in eight days is given. The total yield during the first three to five hours after the injection was 109.3 grams, the average "purgation" therefore amounted to about 7 grams in three to five hours. Normal rabbits frequently pass as much as that and more in one to two hours (see Tables A, B, C, D); the importance of this striking variability in the fecal output of normal rabbits will be pointed out a little more fully when the question of controls is taken up. Under these conditions, is it justifiable to consider such a meager amount of scybala the result of a purgation especially as *normal*

¹ *Ibid.*, p. 194.

² *Ibid.*, p. 194.

³ Bancroft: *loc. cit.*, p. 196.

rabbits often pass much more in a shorter time? I do not think so. By purgation something perfectly obvious is meant, something which does not require statistical research for identification. As a definition the one I gave in a former paper should, I think, still hold: "By purgation is here understood the passage of soft and unformed feces in amounts exceeding that which normal animals might conceivably pass;"¹ for apparently normal animals at times pass some soft and unformed feces in small amounts. With this definition, which embodies the usual conception of a purgation, there need hardly be any doubt about the purgative effect of any injection.

Bancroft's statement² that the amount of feces eliminated in a certain time, *irrespective of consistency*, must be considered in determining whether or not "purgation" has occurred, is admissible only if at least four control rabbits are kept. The reason is this: normal rabbits do not pass equal amounts of feces during the same time; certain rabbits may pass nothing during the day while during the night a normal quantity is eliminated; or most of the scybala are passed during the day, and only few during the night; or approximately equal amounts are passed during the day and night. The same rabbit may show the above variation at different times. There are still other variations, an apparently normal rabbit may be constipated for no definite reason for a day or more, at irregular intervals. Most of these fluctuations are brought out in Tables A, B, C, D. Bancroft's tables also show many of these variations very well;³ (see Table I, p. 195, Rabbit 3; Table II, p. 197, Rabbits 3 and 4; Table III, p. 200). These large variations among normal rabbits make a number of controls imperative, if moderate accuracy is desired; otherwise the results would surely deceive, for a "purgation" in Bancroft's sense can easily be figured out from the normal output of five rabbits (Table C). If Rabbit VIII had received an injection, for instance, the results would have simulated a well-marked "pur-

¹ Auer: *loc. cit.*, p. 17.

² Bancroft: *loc. cit.*, p. 192.

³ Bancroft also recognizes fluctuations in the daily output (p. 196), but seems to think that these variations affect the controls in the same way at the same time.

gation." On the other hand, if the ordinary criteria of a purgation are accepted, no such mistake could be made.

Bancroft's Table II, p. 197, furnishes the proof that sodium citrate injected subcutaneously in double and treble the quantity employed by MacCallum, not considering even the stronger concentration, does *not* purge. A résumé of the table is here reproduced.¹

Day.	Hour.	No. 2. 1501 grams subcutaneous.		No. 3. 1697 grams control.	No. 4. 1621 grams. control.
		Dose.	Feccs.	Feces.	Feces.
		cc.	grams.	grams.	grams.
1	First 1 hour.....	30	13.8	8.3	2.4
	" 3 hours		21.6	14.9	5.9
2	First 1 hour.....	30	0.0	2.3	4.3
	" 4½ hours.....		8.7	23.8	5.5
3	First 1 hour.....	30	8.8	7.8	8.8
	" 4 hours		18.2	24.8	18.1
4	First 1 hour.....	30	10.0	2.1	13.4
	" 4 hours		21.0	16.1	32.9
<i>Totals:</i>					
	1st hour.....		32.6	20.5	28.9
	3-4½ hours.....		69.5	79.6	62.4

Animals were fed hay, grain and water.

It will be seen that in every experiment except the first, one or both of the controls at all times passed at least as much as the injected animals. *Three out of four experiments therefore were against the view Bancroft set out to prove*, that sodium citrate purges when given subcutaneously. Yet in spite of this markedly negative result, Bancroft ventures to make this misleading statement, though it is literally correct in a way: "It is only by comparing the totals that it can be seen that in spite of the fact that the controls are larger animals" (controls are 1-200 grams heavier) "and pass more feces, yet during the first hour after the administration of the citrate the experimental animals passed the greater amount of feces."² Now this holds true of the first hour *total* only, and not of the individual experiments. The method of comparing totals and averages, which Bancroft uses extensively in his paper,

¹ Accentuation of some of the figures due to the writer.

² Bancroft: *loc. cit.*, p. 198.

is methodologically wrong and gives false impressions when applied to a few experiments; it is safe to use only when large numbers of data are at hand. On the whole however the experiments did not seem satisfactory to Bancroft; he says that as the daily output of the rabbits increased greatly, due to their diet of hay, grain and water, "it is not surprising that the effects of small doses of sodium citrate might be hard to detect."¹ *Bancroft therefore admits that under some perfectly normal conditions rabbits show no purgation after the subcutaneous injection of "small doses" of sodium citrate; for a "purgation" which is "difficult to detect" has not even an academic interest.* Regarding the doses, let me point out again that Bancroft's "small doses" are two to three times larger than those employed by MacCallum, moreover they are also of a higher concentration.

Sodium citrate is the only ordinary saline which Bancroft employed in doses short of enormous, and even this salt he only used subcutaneously; none was given intravenously in moderate doses. Barium chloride, whose purgative action is doubted by no one, was tested; in this connection it seems necessary to state that barium chloride is not to be classed among what are ordinarily called the saline purgatives, and no other writers, as far as I am aware, so classes it.

Some New Experiments.

Although the above analysis of Bancroft's experiments and arguments shows clearly the incorrectness of his claim, a new series of experiments was carried out in which only sodium citrate in $\frac{M}{8}$ solution was injected, special attention being paid to the controls and to the hourly output. The following tables also illustrate the fluctuations which normal rabbits show and to which perhaps sufficient attention has already been called. A few additional words may, however, be permissible. The series of tables appended represent only a few of those obtained from nine rabbits observed in two divisions for twenty-four days. The rabbits in each division were approximately of the same size, and averaged 1400 and 2400 grams. At the end of the period of observation they had all gained in weight, with the

exception of Rabbits I and IX. They were all fed the same amount and kind of food at the same time, and were confined in separate cages in the same room.

TABLE A.

July 29.

Time.	I. White and gray rabbit, male.	II. White and black, female	III. Black, male.	IV. Gray, female.
8 to 9 a. m.	1 pellet	0 feces	0 feces	0 feces
12 m.	3 grams	2 grams	13 grams partly soft	12 grams
1.30 p. m.	2 pellets	7 "	11 grams	10 "
2.30 "	1 pellet	4 "	5 "	5 "
3.30 "	2 pellets	2 "	4 "	5 "
4.30 "	5 grams	2 "	9 "	5 "
5.30 "	5 "	4 "	2 "	1 "
to 8 a. m., July 30	5 "	12 "	9 "	27 "
Total, 24 hrs.	19 "	33 "	53 "	65 "

8 a. m., fed wet oats

4 p. m., fed cabbage leaves.

Average weight, 1400 grams

} Equal amounts to each rabbit.

TABLE B.

August 20.

Rabbit.	V. White, female	VI. Gray, female.	VII. Gray, male.	VIII. Gray, male.	IX. Gray, male.
9 to 10 a. m.	0 feces	0 feces	0 feces	0 feces	0 feces
11 "	0	0	4 grams	some soft 2 gram	0
12 m.	0	2 pellets	2	0	0
1 p. m.	0	0	1	5 gram	2 pellets
2 "	0	0	3	2.5	0
3 "	0	0	0.4	5.5	0.6
4 "	0	0	(2 pellets) 0.4	0.7	0
5 "	0	0	1.5	1 pellet	0.1
6 "	0	1 pellet	0	0	2
to 9 a.m., Aug. 21	18.5	22	29	21.5	21.5
Total, 24 hrs.	18.5	22	40	37	24

9 a. m., fed wet oats.

5 p. m. cabbage and carrot greens.

Average weight, 2400 grams.

} Same amount to each rabbit.

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TABLE C.
August 21.

Rabbit.	V.	VI.	VII.	VIII.	IX.
9 to 10 a. m.	0 feces	1.5 gram	2 gram	some soft 12 gram	1.5 gram
11 "	0	1.5	0.5	1.5	0 irregular shape
1 p. m.	0	0	0.5 some soft	12	1
2 "	0	1 pellet	2 soft	9	1
3 "	0	1	1.5 irregular shape	0	1
4 "	1.5 gram	0	2	0	0.5
5 "	0	0	1.5	0	0.6
6 "	0	0	2	1	2.5
to 9 a. m., Aug. 22	26	33	1.5	21.5	13
Total, 24 hrs.	27.5	37	13.5	57	21

9 a. m., fed wet oats.

5 p. m. cabbage and carrot greens.

TABLE D.
August 22.

At 11.15 a. m. Rabbits V and VII received 15 cc. $\frac{M}{8}$ sodium citrate solution subcutaneously, lumbar region.

Rabbits.	V.	VI control.	VII.	VIII control.	IX control.
9 to 10 a. m.	0 feces	0	0	3.5 grms.	0
11 "	0	0	1 pellet (1 pellet)	9	0
12 "	0	0	0.1	5.5	0
1 p. m.	0	0	0	4	0
2 "	5	0	1	2	0
3 "	3	(1 pellet) 0.1	1.5	(1 pellet) 0.1	0
4 "	2	0.9	0	0	0
5 "	2.5	1	0	0	1
6 "	0	0	1 soft	0	3.5
to 9 a. m., Aug. 23	13	4	3.5	11	9
Total, 24 hrs.	25.5	6	7	35	13.5

9 a. m., wet oats.

5 p. m., cabbage and lettuce.

On August 22 (Table D) Rabbits V and VII received subcutaneously 15 cc. of an $\frac{M}{8}$ sodium citrate solution at 11:15 a.m. Nothing resembling a purgation resulted: Rabbit VII passed 1.2 grams and Rabbit V 5 grams during three hours after the injection. *Control VIII passed 11.5 grams in the same length of time*; Controls VI and IX passed practically nothing being in a stage of physiological constipation just as the injected Rabbit VII was. This experiment illustrates well the danger of too few control rabbits; if control Rabbit VIII had been absent a meager "purgation" would have resulted on paper, especially if averaged.

To sum up the above considerations of Bancroft's first group of experiments:

(1) Bancroft did not repeat MacCallum's work, for he used much larger doses.

(2) His results do not show that the injections bring on any purgation.

(3) A new series of experiments confirmed completely my former results, at least as far as sodium citrate is concerned. The fallacy is pointed out in using only one or two control rabbits in experiments of this nature.

Bancroft's Second Group of Experiments.

The second group, excluding barium chloride, deals entirely with the effects of *toxic* and *lethal* doses of some saline purgatives. These experiments are not relevant to the question at issue, for it is obvious that the purgative injections must be harmless, just as harmless as when administered *per os*. This was fully realized by MacCallum, for otherwise he certainly would not have advised that "the subcutaneous or intravenous administration of some of the salts, especially sodium citrate, might be safely resorted to"¹ in human beings. In spite of this, these experiments will be considered, for they throw light on Bancroft's methods and reasoning. For instance, in order to obtain fluid feces by the intravenous method, he injected usually about 400 cc. of an $\frac{M}{8}$ sodium sulphate solution through the marginal

¹ MacCallum: *loc. cit.*, p. 108-109.

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ear vein.¹ This quantity corresponds approximately to four times the entire quantity of blood which an average rabbit possesses. After flooding the vascular system with such an amount of liquid it is not surprising that he obtained fluid feces from the animals which did not succumb. But it is surprising that Bancroft considers this an indication that sodium sulphate purges when injected intravenously, for 0.6 per cent sodium chloride solutions when injected in large amounts into the bloodstream cause a greatly increased fluidity of the gut contents, as was well shown in the famous experiments of Cohnheim and Lichtheim in 1877.² These authors state that under the conditions mentioned "the lumen of the gut always contains a very considerable quantity of a thin more or less fecal fluid." It is evident that Bancroft's experiment proves nothing regarding a distinctive purgative effect of sodium sulphate *per se* when injected with enormous quantities of liquid into the circulatory system. That Bancroft did not arrive at this conclusion is probably due to his controls. It seems that he considered perfectly normal rabbits controls for these experiments, for nowhere does he mention that his controls received an equal infusion of physiological saline solution. But a normal rabbit, surely cannot be considered an adequate control for an experiment in which the vascular system of the experimental animal is so swamped with fluid that "œdematous, jelly-like masses in the vicinity of the kidney"³ are found. If proper controls had been kept; that is, controls which received an equal quantity of normal physiological saline solution intravenously, Bancroft probably would have been able to corroborate Cohnheim and Lichtheim's findings and therefore would have hesitated to ascribe a purgative action to sodium sulphate *per se* when injected intravenously in this manner.

The doses employed for subcutaneous injections were enormous: for instance, sodium sulphate was injected in amounts

¹ Bancroft: *loc. cit.*, p. 201, 206.

² Cohnheim and Lichtheim: *Virchow's Archiv*, lxi, p. 121, 1877.

³ Bancroft: *loc. cit.*, p. 204. These œdematous masses Bancroft considers "direct evidence" that the urine carried with it much of the sodium sulphate injected.

varying from 60 to 110 cc. of an $\frac{M}{2}$ (16 per cent!) solution.¹ This would correspond for a 70 kilogram individual, to 3900 cc. or to 624 grams of the crystalline sodium sulphate. No wonder that "the injected animals were seriously injured and often killed"² by these doses. Most practitioners will probably hesitate to give over a pound of sodium sulphate subcutaneously in order to obtain a purgation.

When sodium citrate was given subcutaneously, almost invariably lethal doses had to be given in the attempt to obtain fluid feces, for in only one case did a rabbit survive a dose greater than 100 cc. of an $\frac{M}{3}$ solution,³ and we may be certain that no smaller dose had the desired effect. On postmortem examination of these rabbits Bancroft found fluid feces in the "large intestine" and argues that they would have been passed had the animals lived a little longer; he does not mention the condition of the intestinal walls.

This second group of experiments shows strikingly that Bancroft was not satisfied with the purgative effect of rational doses of salines when injected subcutaneously or intravenously, and really was forced to use these tremendous doses in order to obtain some approximation to the result obtained when the salines were given by mouth.

Magnesium sulphate.—In an appendix to his article, Bancroft extends his criticism to a paper by Dr. Meltzer and the writer, which deals with the relation of magnesium sulphate and chloride to intestinal peristalsis.

This and other researches had been undertaken on the basis of a hypothesis that all magnesium salts exert an inhibitory effect. In agreement with this hypothesis it was found that both magnesium sulphate and chloride, when injected subcutaneously or intravenously, did not cause intestinal peristalsis or purgation. Moreover, these injections directly inhibited existing peristalsis. Now MacCallum states that magnesium sulphate when injected subcutaneously or intravenously causes purgation,⁴ and explains this action by the statement that intestinal peri-

¹ Bancroft: *loc. cit.*, p. 200.

² Bancroft: *loc. cit.*, p. 199.

³ Bancroft: *ibid.*

MacCallum: *loc. cit.*, p. 108.

stalsis is stimulated. In commenting on this difference between MacCallum and ourselves with regard to magnesium sulphate, Bancroft speaks of a "misconception" (p. 208) of Dr. Meltzer and the writer in believing that MacCallum ascribes the purgative action of this salt to the magnesium which it contains; he also offers a theory of its action, according to which the SO_4 ion stimulates and the Mg ion inhibits, thus producing peristalsis and purgation. The difference however is not one of theory but of fact; what we stated were facts based on numerous observations. On the basis of still more numerous observations we may repeat that magnesium sulphate as well as magnesium chloride do not purge when injected subcutaneously or intravenously.

Bancroft apparently made no observations of his own with magnesium sulphate; he merely offered a theory. If he wishes to oppose his theory to our facts, we shall not argue with him. We wish however to disagree emphatically with an important part of his theory, that "calcium inhibits more strongly than magnesium" (p. 209). The reasons for this disagreement will be given in communications shortly to be published.¹

Bancroft's Disagreements with MacCallum.

As Bancroft's avowed purpose² is to corroborate the results of MacCallum's experiments, it may be not out of place to call attention to some discrepancies in the unanalyzed testimony itself.

To illustrate:

(1) MacCallum found that the subcutaneous injections of small doses of salines *constantly* increases two to sixfold the amount of feces eliminated during the following time interval.³

Bancroft's data for Table II force him to admit that under

¹At the meeting of the American Physiological Society in Chicago, December, 1907, and also at the December meeting of the Society of Experimental Biology and Medicine, experiments were demonstrated which conclusively showed the antagonistic action of calcium to the inhibitory action of magnesium. Moreover, calcium hastens and magnesium delays the onset of rigor mortis (*Journ. of Exp. Med.*, x, p. 45, 1908).

² Bancroft: *loc. cit.*, p. 191.

³ MacCallum: *loc. cit.*, p. 103.

some perfectly normal conditions the purgative effect of those injections could not be detected.¹

(2) MacCallum states that "a much larger dose" is required to produce the same effect when the salines are introduced into the stomach or intestine than when injected subcutaneously or intravenously.²

Bancroft, on the other hand, devotes much space to an explanation why sodium citrate and sulphate are "so much more effective" in producing fluid feces when given *per os* than when injected subcutaneously or intravenously.³

(3) MacCallum noticed that the feces were sometimes semi-fluid after the subcutaneous injection of small doses of salines, and calls particular attention to this.⁴

Bancroft could not obtain this increased fluidity;⁵ moreover, he states definitely that "the action of small doses of weak purgatives [the salines] is to increase only the *amount* of feces eliminated * * *"⁶ differentiating clearly in the preceding sentences between amount and consistency.

These differences alone should have prevented Bancroft from stating, without any modifying clause, that "MacCallum's results have been confirmed in every respect," as he does repeatedly.⁷ What analysis of Bancroft's work does regarding a corroboration of MacCallum's work has already been shown.

SUMMARY.

Moderate doses of the saline cathartics exert no purgative action when injected subcutaneously or intravenously.

Large doses similarly administered are dangerous and often fatal.

When given in large quantities intravenously, the fluid feces resulting cannot be attributed offhand to a purgative action of

¹ Bancroft: *loc. cit.*, p. 198.

² MacCallum: *loc. cit.*, p. 102-103, p. 106.

³ Bancroft: *loc. cit.*, p. 199, pp. 201-204.

⁴ MacCallum: *loc. cit.*, p. 103.

⁵ Bancroft: *loc. cit.*, p. 196.

⁶ Bancroft: *loc. cit.*, pp. 191-192.

⁷ *Ibid.*, p. 193, p. 206, p. 210, p. 211.

the salt injected, the increased fluidity of the gut contents being due partly or entirely to an increased secretion and transudation of the glands of the gut in their attempt to aid the overwhelmed kidneys. Physiological saline solution infused in large quantities has the same action (Cohnheim and Lichtheim).

Normal rabbits show such marked variations in the total hourly output of feces that at least four controls should be used in testing for the minimal purgative effect of any substance.

Bancroft's statement that controls in my previous experiments would have demonstrated a purgation in the experimental animals is unintelligible, for a majority of those rabbits passed no feces for one hour after injection of the salines;¹ controls could not do less.

Bancroft's own work does not justify his own conclusions, nor does it corroborate MacCallum's results:

(a) The calculation that the subcutaneous injection of small doses of sodium citrate increases the fecal output to twenty-three times that of normal animals is wrong, for it is based on the output of artificially constipated controls.

The passage of a few grams of dry feces, in amount well within the limits of normal variations, should not be called a purgation (13 out of 16 of his experiments showed less than 8 grams of feces in 3 hours); such a "purgation" has neither scientific nor practical interest.

(b) His second series with subcutaneous injections of small doses demonstrates no purgation whatsoever.

(d) The subcutaneous or intravenous injection of toxic and lethal doses of the salines is irrelevant to the question at issue, as obviously the injections must be as harmless as when administered by mouth.

Note added at proof correction.

While this article was in press, a paper by Frankl, from Hans Meyer's laboratory, appeared in the *Archiv für experimentelle Pathologie und Pharmacologie* (lvii, Heft 5 u. 6, p. 386) which deals with the effect of sodium sulphate when injected intravenously. Frankl observed no purgation, but rather a moderate constipation, a result perfectly in accord with mine.

¹ According to MacCallum (*loc. cit.*, p. 103) the purgation usually occurs during the first hour.

SERUM TREATMENT OF EPIDEMIC CEREBRO-SPINAL MENINGITIS.*

By SIMON FLEXNER AND J. W. JOBLING.

(From the Rockefeller Institute for Medical Research, New York City.)

INTRODUCTION.

During the prevalence of the epidemics of cerebro-spinal meningitis in America and Europe from 1904 to 1907 *Diplococcus intracellularis*, discovered by Weichselbaum in 1887, was established finally as the cause of epidemic meningitis. In the course of the studies of this microorganism carried out by one of us (Flexner¹), as one of a commission appointed by the Department of Health of the City of New York to investigate epidemic meningitis, an attempt was made to modify favorably the course of experimental infections with the diplococcus in animals by antisera prepared in several kinds of small animals from *Diplococcus intracellularis*. The ultimate purpose of these experiments was the employment of an antidiplococcus serum in the human infection once it was shown that it could be effective in the experimental infections. Flexner's first reports established that guinea pigs and monkeys, in which the conditions of the infection could be controlled, can be saved from otherwise fatal effects of the diplococcus by the use of the antiserum. Up to the time the first report was published a sufficient opportunity to test an antiserum in human beings had not appeared. Since then a diplococcus antiserum prepared by us in the horse has been tested upon several series of cases of epidemic meningitis, occurring in New York, Philadelphia, Cleveland, Castalia and Akron, Ohio, Edinburgh, Scotland, and Belfast, Ireland. The report which follows deals exclusively with the results of the use of the antiserum in human beings affected with epidemic meningitis.

* Received for publication November 9, 1907.

¹ *Jour. of the Amer. Med. Assoc.*, 1906, xlvii, 560. *The Jour. of Exper. Med.*, 1907, ix, 168.

The tests of the antiserum upon which this report rests could not have been carried out without the cordial coöperation of a considerable number of physicians who showed great interest in the undertaking. It will not be possible for us to thank personally, or even by name, all those participating in the tests and we will have, therefore, to content ourselves with the mention of those physicians who were very active in carrying them out. To Dr. L. W. Ladd, of Cleveland, who carried the antiserum to Castalia and Akron, Ohio, and who was the first to employ it systematically in a series of cases of meningitis, we feel an especial and deep obligation, on account of his early interest and the difficulties which he encountered in Castalia in following the cases which arose at widely separated points in a sparsely-settled country district. We are also grateful to Dr. Crile, of Cleveland, who brought to our attention the epidemic at Castalia and selected Dr. Ladd to administer the serum, to the physicians of the City Hospital of Akron, for their interest in the subject and the full reports which they supplied, to Dr. W. T. Longcope of the Pennsylvania Hospital, and Dr. B. F. Royer of the Municipal Hospital, Philadelphia, to Dr. L. Emmett Holt, of the Babies Hospital, and Dr. Strain of St. Vincent's Hospital, New York City, to Dr. Harvey W. Cushing, of Baltimore, Dr. Claude B. Ker, of Edinburgh, and Dr. A. Gardner Robb, of Belfast, and to the attending staffs at the several hospitals who permitted the trials to be made on their patients.

There will now follow the records of the cases of epidemic meningitis treated with the serum which are presented, with a few changes, in the precise form in which they came to us. The only alterations made in the reports consist of abbreviations of the hospital records where certain details could be omitted with a view of saving space and time, and the addition, at the end of each case, of a brief discussion, the purpose of which is to re-present the salient features with especial reference to the influence on the course of the disease exercised by the lumbar punctures and serum injections.

We regret that in some instances we have not yet received the full reports of cases treated with the antiserum, and are, therefore, restricted to the use of brief statements given in letters to one of us

(Flexner). These omissions are at present unavoidable and have been brought about by the great distances from New York at which antiserum is being tried, or by other circumstances which have temporarily caused withholding of the records. We believe that should the epidemic in America suffer a recrudescence the antiserum will receive a larger and more searching test; and, in any case, since the disease is still appearing sporadically over a wide territory in America and threatens to reappear in force in Great Britain, we expect to be able to publish a second and more complete report on the serum treatment of epidemic meningitis at no very distant date.

THE EPIDEMIC OF CEREBRO-SPINAL MENINGITIS AT AKRON, OHIO.

The epidemic at Akron began in April, 1907, and embraced about twenty cases of meningitis. We are greatly indebted to Dr. W. S. Chase for many of the facts on which are based our consideration of this epidemic. Between May 9 and June 16 there were reported to the health officer, as having been treated outside the hospital, nine cases of meningitis of which eight died and one recovered. Dr. Chase states that the patient who recovered presented atypical symptoms and no bacteriological examination of the spinal fluid was made. None of these cases received the antiserum.

Eleven cases of epidemic meningitis, established as such by symptoms and by bacteriological examination, were treated in the City Hospital with the antiserum. Eight of the cases recovered and three died. The period of first injection of the antiserum, the total amount of antiserum injected, and the mode of termination of the disease in the eleven patients are tabulated below.

Case	I, 1st injection	5th day;	total serum injection	82.5 c.c.; recovery by lysis.
" II.	"	30th hour;	" "	10.0 c.c.; died.
" III.	"	12th day;	" "	20.0 c.c.; recovery by lysis.
" IV.	"	2d day;	" "	43.5 c.c.; recovery by lysis.
" V.	"	7th day;	" "	25.0 c.c.; recovery by crisis
" VI.	"	2d day;	" "	22.5 c.c.; died.
" VIII.	"	1st day;	" "	35.0 c.c.; recovery by crisis
" VII.	"	1st day;	" "	15.0 c.c.; died
" IX.	"	2d day;	" "	105.0 c.c.; recovery by lysis
" X.	"	1st day;	" "	25.0 c.c.; recovery by crisis
" XI.	"	2d day;	" "	22.5 c.c.; recovery by crisis

Two of the fatal cases were of the fulminating type, and one (Case VI) was injected first with the serum at the end of the second day and again on the fourth day of the illness and died three hours after the second injection. The fulminant cases died six hours and ten hours respectively after the first serum injection.

The total number of cases treated with the antiserum at Akron being eleven it is obvious that little value can be attached to the results stated in percentages. However, the following comparison may be made.

Nine cases of meningitis untreated with the antiserum: Eight or 89 per cent. died and one or 11 per cent. recovered.

Eleven cases treated with the antiserum: Eight or 72 per cent. recovered and three or 27.3 per cent. died.

Eliminating from the calculations the fulminating cases as being beyond reach of treatment, the figures obtained are:

Nine cases treated with the antiserum: Eight or 89 per cent. recovered and one or 11 per cent. died.

City Hospital, Akron, Ohio, Service of Dr. W. S. Chase.

CASE I. V. H. White female, aged 12 years. School girl.

Present Illness.—At 1 a. m. April 28, patient complained of pain in legs and stomach; at 4 a. m. became nauseated and vomited. She remained in bed during the greater part of the day and vomited frequently. Late in afternoon arose and walked out for 15 minutes. At 7 p. m. she became unconscious and voided urine involuntarily. She was restless and noisy until 2 a. m., April 30, when she slept for 2 hours; then the restlessness returned. Rigidity of neck and slight retraction of the head were first noticed on the 30th instant. Admitted to hospital that day.

Physical Examination.—Patient unconscious and restless and screaming, neck rigid, head retracted, pupils equal and react to light, patellar and abdominal reflexes absent, Kernig's sign marked, Babinski not present, herpes on lips. Temperature 99.6°, pulse 100, respiration 24.

May 1. Totally unconscious, restless and noisy; temperature ranged from 99.6° to 102.2°; pulse from 92 to 160.

May 2, 10 a. m. Lumbar puncture, 15 c.c. of opalescent fluid withdrawn. Microscopical examination showed Gram-negative diplococci within and outside of pus cells. Temperature from 99.6° to 102°.

May 3. Condition unchanged.

May 4, 6 a. m. Temperature 101.8°.—8.30 a. m. 10 c.c. opalescent fluid withdrawn by lumbar puncture and 10 c.c. *antimeningitis serum injected*.—10 a. m. Temperature 100°.—10.40 a. m. Convulsion involving face, eyes and left hand lasting two minutes.—11.21 a. m. Second convulsion, which continued until relieved with chloroform.—1.50 p. m. Continuous nystagmus of both eyes; twitch-

ing of arms and upper lip; reflexes of extremities and cornea absent.—3 p. m. Convulsions continue except when controlled with chloroform. Kernig's sign more marked than before; rigidity of neck increased; convulsions controlled with chloroform until 5.30 p. m., nystagmus continuous between convulsions.—6 p. m. Temperature 102.2°.—8 p. m. 99.8°.—8.45 p. m. Conscious.—9.30 p. m. Convulsion controlled with chloroform.—12 midnight. Temperature 98.2°.

May 5. Temperature did not rise to 100° until 9 p. m. Patient slept from 12.30 a. m. to 3 a. m. Still some twitching of mouth and left eye. At 4 a. m. took water on being aroused. At 6 a. m. complains of thirst and asks constantly for water and milk.—7 p. m. The patient has taken freely of milk and water during the day and cries for food. She has rested well until midnight. Temperature below 100° until 9 p. m., when it was 100°, and at midnight 101.4°.

May 6. Rested fairly well; condition improved; took considerable nourishment.

May 7 and 8. No essential change.

May 9. Restless after midnight, complains bitterly of frontal headache; temperature rose at 12 m. to 102.8°, and at 3 p. m. to 103.4°.—9 p. m. Temperature 103.8°; lumbar puncture under chloroform anæsthesia, 30 c.c. opalescent fluid withdrawn and 7.5 c.c. *antiserum* injected.—10.30 p. m. Temperature 101.8°.—12 midnight. 101.6°.

May 10, 2 a. m. Slight twitching of hands; vomited. Rested well and took nourishment during the day; temperature normal to subnormal.

May 11. Temperature remained below 100°; patient rested well.

May 12, 3 a. m. Temperature 100°; very restless and noisy. Temperature rose during the day.—9 a. m. 102.8°.—12 m. 103.6°.—9 p. m. 104.2°. Withdrew, under chloroform anæsthesia, 60 c.c. opalescent spinal fluid, in which diplococci were not found on microscopical examination, and injected 7.5 c.c. of the *antiserum*.—12 midnight. Temperature 102°.

May 13. Patient had a good day and the temperature was 100° at 6 a. m., normal at noon and 97° at midnight.

May 15. Complaints of bad headache; temperature rose in afternoon to 104° (3 p. m.).—9 p. m. Temperature 103.2°, lumbar puncture yielding 22 c.c. opalescent fluid free of diplococci.—12 m. Temperature 103.8°.

May 16. Temperature remained above 100° until 9 p. m. Vomited several times; very restless. The condition fluctuated, but remained essentially unchanged until May 25. The rigidity of the neck continued and Kernig's sign was still present and the reflexes had returned in some degree. Pain was still complained of in the head and other parts. The temperature fluctuated between 99° and 102.5°. At 11 a. m. 90 c.c. of less opalescent fluid were withdrawn by lumbar puncture and 20 c.c. of *antiserum* injected. The spinal fluid contained leucocytes but no diplococci. At 8 p. m. marked urticaria developed. The patient vomited and was restless and noisy during the afternoon. The temperature fell and reached normal at 12 p. m. the next day. The temperature remained at normal or a little below for four days, then rose to 101.8°, and fell in a few hours. The general condition was better.

June 1. Herpes appearing on lips; neck rigidity lessened; Kernig's sign still marked. Temperature fluctuated between 99.4° and 101°.

June 3. Restless, noisy, complains of pain. Temperature rose suddenly at

9 p. m. to 103°.—11 p. m. Lumbar puncture under chloroform anæsthesia. 75 c.c. of fluid withdrawn and 15 c.c. of *antisera* injected. The spinal fluid showed on microscopical examination a few diplococci and a very small number of leucocytes. Temperature at midnight 100.4°. The temperature was normal June 4, and did not rise above again. The note on June 9 reads: "Patient's condition greatly improved; she is rational and quiet all the time, the reflexes are normal, Kernig's sign is absent, the rigidity of the neck has disappeared, there is complete absence of all pain, and all the functions appear to be normal. The patient states that she feels well."

June 10 to 14. The note states that the temperature has remained practically at normal; sleep is good, but there is some complaint during the day of pain in various parts; the reflexes are normal and Kernig's sign is absent, but there is involuntary movements of bowels and bladder.

June 15. The note states that the patient still complains of pain and is very restless and noisy. The temperature is about normal. 2 p. m. Headache and vomiting. At 3 p. m. lumbar puncture was performed and 75 c.c. of spinal fluid were withdrawn. Microscopical examination showed "many extracellular diplococci and no leucocytes." 22.5 c.c. of *antisera* injected. The next notes are given entire.

"From June 17 to 25, inclusive, the temperature ranged from normal to 101.6°. The latter temperature was reached on the 18th instant; after that it did not again reach 100°. Appetite was good. Pain in the ear and abdomen complained of responded to palliative treatment. Involuntary micturition and bowel movements continued. On the 25th the patient sat up in bed without discomfort."

"Beginning June 26 (58th day of disease) the temperature remained normal until the day of discharge (96th day of disease). After June 28 the involuntary movements ceased. . . . On 96th day the patient was examined and found well; discharged. She reports to hospital twice weekly, and she is in excellent condition."

Discussion.—This case is one of severe and protracted epidemic meningitis. The diagnosis is clearly established by the symptoms and the bacteriological examination. The special interest which the case has for us is involved in the question whether its course was essentially influenced by the several injections of antimeningitis serum. It does not seem possible to give a definite and outright answer to this question. On the other hand the following points appear to be clear: The first injection of serum (on May 4) was followed by severe convulsions enduring during a large part of the day and requiring to be relieved by chloroform. The convulsions did not re-appear spontaneously and were not excited by subsequent injections of larger amounts of the serum. It is probable, therefore, that the association was accidental. The serum injections generally were followed by a fall in the temperature which reached

or approached the normal and remained at these levels for several days. The temperature and symptoms were subject to much fluctuation, but when the former rose approximately to 104° and the latter became severe, lumbar puncture and serum injection were followed by a tolerably prompt improvement in the patient's condition. On one occasion (May 15), lumbar puncture was performed and no serum injection made and it is noteworthy that the temperature did not fall as in the instance in which the serum injection followed the puncture. The condition of the patient becoming unsatisfactory on May 25 a puncture and serum injection being carried out the temperature fell promptly, remained at or about normal for several days, and the patient's general condition was described as improved. These different results may mean nothing actually, but we put them together since it is desirable to secure light on the independent effects of the puncture alone and the puncture plus the serum injections. Note should be taken of the occurrence of urticaria following the serum injection of May 25. The clearing up of the cerebro-spinal fluid, following the puncture and serum injections, and the disappearance from it of many leucocytes and all *demonstrable* diplococci before the subsidence of the symptoms is shown to be possible. The final history of the case indicates that there may still remain an active focus in the membranes from which a fresh invasion of diplococcus into the spinal fluid may take place, with which is associated a reappearance of certain symptoms and a sudden rise in temperature. Such a relapse would seem to have occurred on June 3 and not to have been attended by a rich outpouring of leucocytes into the cerebro-spinal fluid. If this observation is admitted it is at least worth noting that the condition was quickly controlled by the puncture and serum injection although it is not established that these means were the sole or chief causes of the abrupt termination of the relapse. Only an accurate and painstaking clinical and bacteriological study of protracted and relapsing cases of epidemic meningitis will suffice to determine the manner in which, and the rapidity with which the body's forces unaided deal with the diplococcus. Total amount of antiserum injected, 82.5 cubic centimeters.

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City Hospital, Akron, Ohio. Service of Dr. George Rankin.

CASE II. H. R. White male, aged 17 years. Rubber worker.

Present Illness.—Admitted May 9, 1907, 12 m. The day before admission the patient ~~was at his work~~ but in the evening he complained of headache and feeling ill, and he went early to bed. At 4 a. m. his family was aroused by his falling out of bed. He was picked up in an unconscious state. He was seen at 9 a. m. by a physician who found him unconscious, restless, throwing himself about, and with a temperature of 104° F. Still unconscious and tossing about on admission to hospital at 12 o'clock noon.

Physical Examination.—Face flushed; perspiring freely; pupils moderately dilated, do not react to light; neck rigid and slight retraction of head; abdominal, patellar and plantar reflexes absent; Kernig's sign present.

May 9, 1 p. m. Temperature 101.6°, pulse 90, respiration 40. Lumbar puncture yielded 60 c.c. of opalescent fluid containing pus cells and extra- and intracellular diplococci.—3 p. m. Temperature 101.6°. Vomited several times during the afternoon; pulse could not be counted during afternoon.—6.30 p. m. Temperature 102.8°.—9 p. m. 103°.—12 midnight. 105.4°, respiration 70. At 11 p. m. lumbar punctured and 60 c.c. opalescent fluid withdrawn and 10 c.c. of antiserum injected. The microscopical examination of the fluid gave the same results as the first fluid withdrawn. The pulse continued uncountable and the respiration high. Temperature at 3 a. m. 106.2°, at 5 a. m. 107.2°; died at 5.42 a. m. May 10.

Discussion.—This case is an example of the fulminating type of epidemic meningitis. From the appearance of the marked symptoms and the death, less than 36 hours elapsed. At the time of the second lumbar puncture and the first injection of the serum the patient was in a critical condition and survived these operations only six hours. It is highly improbable that the serum could influence favorably so severe an infection as existed in this case, but it would have been proper to have injected it earlier—immediately after the first lumbar puncture had established the diagnosis—and in much larger quantity. The reason that the serum was not injected earlier is found in the fact that it had first to be brought from Cleveland; and that the dose was small is explained by the date of the serum's employment, for at that time it was being used very cautiously since we had not yet learned that it could be injected in much larger amount with impunity into the inflamed spinal canal of human beings. Total amount of antiserum injected, 10 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE III. B. S. White male, aged 19 years. Rubber worker.

Present Illness.—Until May 11, 1907, the patient was well. On that day he felt badly, went to bed early and was said to have had a chill during the night. No further history of illness until May 13, when a physician saw the patient who was feverish, restless and complained of pain in head and neck. That night he became unconscious; admitted to hospital next morning (May 14). He was, at that time, unconscious and irrational and very restless, tossing and talking continuously. "Temperature 99°, pulseless at wrist, respiration 32 and shallow, cyanosed."

Physical Examination.—Unconscious and greatly cyanosed young man. Heart beats 56 per minute. Neck somewhat rigid and painful on being moved. Reflexes diminished.

May 14. Temperature varied from 99° to 101.6°. Under stimulants, pulse improved; at 12 p. m. 104, at 12 a. m. 88.

May 15 and 16. Condition essentially unchanged.

May 16. Reflexes absent from extremities and abdomen; neck rigidity marked; Kernig's sign present. Lumbar puncture unsuccessful. Restless and talking.

May 23. Note states: "Patient semi-conscious part and conscious other part of the time. The reflexes, except the plantar reflex, are absent. Neck rigid and painful on being moved. Temperature fluctuated from 99° to 102.4°. Slept and rested well and took considerable nourishment."

May 24. Temperature rose to 103.4°; otherwise no change.

May 26. No marked change. Kernig's sign present; reflexes absent. Temperature at 12 p. m. 103.6°. Lumbar puncture performed under local anæsthesia and 60 c.c. opalescent fluid obtained and 20 c.c. *antiserum injected*. The examination of the spinal fluid showed pus cells and extra- and intracellular diplococci.—3 p. m. Temperature 103.2°.—6 p. m. 102.8°.—9 p. m. 102°.—12 a. m. 99.8°. Patient rested well, perspired freely and took considerable nourishment during the night.

May 27. Temperature ranged from 98° to 99.2°. The note states: "Mental condition improved; reflexes not changed; slept well and took considerable nourishment."

May 28. Temperature ranged from 97° to 97.8°. The note states: "Reflexes are now normal; the mental condition is good, but the neck is still somewhat rigid and slightly painful on being moved."

The next note states that from May 29 to June 6, the date of discharge from the hospital, the patient's condition continued to improve and there was complete recovery.

Discussion.—This case is an example of epidemic meningitis with severe onset and gradual subsidence of symptoms by lysis. The indications are that the patient's recovery was reasonably assured before the successful lumbar puncture and injection of the serum on the twelfth to the fourteenth day of the disease. Until the day of injection the temperature had not remained continuously, during any

one day, below 101° . Within twelve hours of the injection the temperature fell below 100° and did not again rise to that point, tending rather to remain somewhat subnormal. Whether this is more than a coincidence cannot be decided now. The quick return of the reflexes following the puncture and serum injection, and the acceleration of the rate of improvement in the patient's mental and general condition, may also be merely co-incidental, but they were sufficiently great to be regarded as noteworthy. Total amount of antiserum injected, 20 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. L. R. C. Eberhard.

CASE IV. B. K. Female, aged 15 years. Factory worker.

Present Illness.—Patient felt well and worked until May 14, 1907. Awoke 6 a. m. May 15 feeling ill and complaining of headache; ate little breakfast; returned to bed, vomited and became unconscious during the day. Physician called; found temperature 101.4° ; patient restless and irritable and crying out with pain on being touched. Admitted to hospital May 16. She was unconscious and tossing about. The abdominal reflex was absent and the patellar and plantar reflexes greatly diminished. The neck was somewhat rigid and attempts to move it were very painful.

May 16, 9 a. m. Temperature 103.8° , pulse 84, respiration 34.—10.30 a. m. Lumbar puncture under chloroform anæsthesia; 45 c.c. opalescent fluid containing pus cells and extra- and intracellular diplococci withdrawn. 15 c.c. of *antimenigitis serum* injected.—12 p. m. Temperature 102° . It ranged during the rest of the day from 101° to 102.6° .

May 17, 3 a. m. Temperature 99.6° ; 10 a. m. 100.8° ; 5 p. m. 102.2° ; 12 a. m. 102.6° . The note states: "Patient slept part of the night, but is restless at times. Knee, plantar and abdominal reflexes are present. There is marked Kernig's sign and no Babinski reflex; no ankle clonus. Restless and noisy from 9 p. m. to midnight."

May 19, 8 a. m. Unconscious and irrational except when spoken to. Herpes on lips. Internal squint of right and left eyes.—7.30 p. m. Lumbar puncture under chloroform anæsthesia; 45 c.c. of opalescent fluid withdrawn and 5 c.c. *antiserum* injected. Slept from 11 p. m. to 5 a. m.

May 21. Condition has remained essentially unchanged. Temperature, 9 a. m., 103.2° .—9.30 a. m. Lumbar puncture: 3.5 c.c. fluid withdrawn and 3.5 c.c. *serum* injected. Spinal fluid shows diplococci.—7 p. m. Condition unchanged.

May 22. Patient irrational and complains more than previously on being moved. She seems not to have control of the arms, although she moves hands, fingers and legs.

May 25. Condition has not changed materially. 9.30 a. m. Lumbar puncture under chloroform; 75 c.c. of opalescent fluid removed; 20 c.c. of *antiserum* injected. Temperature during the day ranged from 99.6° to 102.8° .

May 26. Temperature ranged from 99.8° to 102° .

May 27. 99° to 102.6° . The note at 6 p. m. states that all reflexes are present, that the left arm cannot be used and the left trapezius is contracted, drawing the

head to the left side. Patient is conscious and rational and rested well. Kernig's sign is present.

May 27. Urticaria has appeared on knees and elbows. The temperature has remained below 100° since 3 a. m.

May 29. Temperature below 100° .

June 1. The temperature has remained below 100° except for one or two brief intervals, when it reached that height. Patient is conscious and rational. All reflexes except the abdominal reflex are present, the neck is less rigid and can be moved voluntarily as can the arms and legs. Kernig's sign still present.

June 5. Temperature has remained below 100° (all temperatures until to-day taken per rectum). Axillary temperature normal.

June 9. Patient improved in every way. Reflexes normal. Kernig's sign absent. Rigidity of neck gone. Has use of all extremities and functions appear to be normal. Urticaria appeared over entire body.

June 10. The final note states that temperature and pulse remained normal and the patient was discharged well on June 27, the forty-first day after admission.

Discussion.—This case is an example of epidemic meningitis with severe onset, moderately prolonged illness, subsidence of symptoms by lysis and complete recovery. The patient was admitted to the hospital about 24 hours after the appearance of the first severe symptoms, and the diagnosis was established by lumbar puncture and a first dose of antiserum administered within the first thirty hours of the disease. No marked or permanent influence on the course of the disease, as far as can be determined, was produced by the puncture and serum, and subsequently three additional injections of serum (following withdrawal of fluid) were made into the spinal canal. On May 25, or approximately the tenth day of illness, an injection of 20 c.c. of serum was given (the two previous injections were of 3.5 c.c. and 5 c.c. respectively). On May 26, the note states that the reflexes, which had previously been absent, had returned, but the same note records the involvement of the trapezius muscle in the rigidity of the neck. May 27 the temperature remained persistently below 100° , and from that date on no rise of temperature above 100° (rectal measurements) was recorded. Urticaria appeared on that day. The condition of the patient improved more or less in the next days and on June 1, the symptoms had considerably abated, voluntary muscular movements had returned and the neck was less stiff. The disappearance of Kernig's sign and the general functional restoration of the body

are noted on June 9 when the patient was regarded as convalescent. It is not possible to assign the specific influence, if any was exerted, of the serum on the progress and final result in this case. Total amount of antiserum injected, 43.5 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE V. F. N. White male, aged 32 years. Laborer.

Present Illness.—The patient had been well, except for coryza, until May 12, when he felt ill and suffered from pain in the muscles and head. May 13 consulted physician who said he had fever and who gave him a purgative and sent him to bed. Did not go to work, felt chilly, vomited, complained of pain in head and neck. Next day no better, but he went to his work and remained until noon. He was obliged to return to bed, where he remained until May 16, when his mental condition becoming alarming he was taken to hospital. Patient became unconscious in the ambulance.

Physical Examination.—Unconscious; neck rigid; reflexes absent; Kernig's sign absent; head slightly retracted; pupils somewhat contracted, react sluggishly to light. Temperature, 4 p. m., 104.2°; 12 a. m. 102.2°. Delirious.

May 17. The note states that the patient was restless, noisy and delirious, and Kernig's sign was now present.—3 p. m. Lumbar puncture attempted, but no fluid was obtained. At 2 p. m., out of bed, delirious, put in restraint.

May 18. Neck very rigid, otherwise condition unchanged.

May 21. The condition fluctuated during the past four days; the temperature ranged from 98.6° to 104.4°, pulse up to 130, respiration rapid and irregular, and there were delirium, noisiness and restlessness. The physical signs have not changed; hiccough has appeared.

May 22, 10 a. m. Under chloroform anæsthesia lumbar puncture was made and 90 c.c. of cloudy fluid, which was under considerable pressure, were removed. At the same time 25 c.c. of antiserum were injected. The spinal fluid showed on microscopical examination pus cells and intra- and extracellular diplococci.—12 m. Temperature 100.8°; pulse 104.—12 a. m. Temperature 102°; pulse 98.

May 23. The note states that the patient rested fairly well after midnight. At 9 a. m. he was conscious and resting quietly and the mental condition was improved. He was free from pain and the reflexes were unchanged. Temperature: 3 a. m. 101.4°, 12 p. m. 100°, 12 p. m. 98°.

May 24. Rested well part of night and day. Conscious and rational. Knee and plantar reflexes present. Kernig's sign still present. Neck still somewhat rigid and painful on being moved. Temperature normal since midnight.

May 25. Temperature normal. The patient has slept well and taken freely of nourishment.

The next note states that from May 25 to June 5 the temperature and pulse were normal; the patient was up and about the ward since June 2, the reflexes were normal, there was no rigidity and no Kernig's sign.

June 6. Patient awoke at 3.30 a. m. complaining of pain in legs and back. He was unable to move his legs and complained of pain when they were moved. Reflexes could not be elicited, but legs were held rigidly. Pain and tactile sensations normal.

June 7. Extremities less painful, but there is marked tenderness over the large nerve trunks.

June 8. Functions of lower limbs gradually returning.

June 12. The patient is improving, the neuritis is diminishing and the extremities can be used.

June 15. Patient discharged cured.

August 2. Patient has reported to the hospital. He is in perfect health.

Discussion.—The onset of the symptoms of meningitis was in this case gradual and extended over 3 days. The symptoms became severe on May 16 and the patient's condition was bad until May 23 when it suddenly and critically changed for the better. From May 23 on the patient's condition continued to improve, with the exception of the discomfort caused by the neuritis which appeared in the legs on June 6 and quickly subsided, until June 15 when he was discharged "cured" from the hospital. The meningeal and mental symptoms and the symptoms of general intoxication were, in this case, profound. On May 22, or approximately the seventh day of the disease, successful lumbar puncture was made and the antiserum injected. Twenty-four hours later the patient's condition was described as improved and the level of the temperature was lower than before. The condition of the reflexes was, however, unchanged. Within forty-eight hours of the puncture and serum injection the patient had become conscious and rational, the reflexes had in part returned, the neck was less rigid, the temperature reached normal, and the patient was resting quietly, sleeping and taking nourishment. No rise in the temperature again occurred. The patient was evidently convalescent. The critical disappearance of the severe symptoms and the lumbar puncture and injection of serum would seem to bear some relation to each other. There was an abrupt transition from a condition of much seriousness, which had endured unchanged for a week, to one of comparative and actual mildness within forty-eight hours of the puncture and injection of the antiserum. Total amount of antiserum injected, 25 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE VI. Z. H. White female, aged 17 years. Rubber worker.

Present Illness.—The patient was at work until May 18, when she went home on account of malaise and pain in back and limbs. At 2 a. m. the next morning

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she complained of severe headache, at 3 a. m. she became unconscious. A physician was called at 7 a. m. (May 19). He said she had fever. During the day she vomited frequently. Axillary temperature, 3 p. m., 104° . Admitted to hospital 6 p. m.

Physical Examination.—Patient admitted in an unconscious state. She is very restless, tosses and rolls her eyes continuously. Knee and plantar reflexes present; abdominal reflex absent; Kernig's sign present.

May 20. Temperature, 9 p. m., 101.2° . Under chloroform anæsthesia 45 c.c. of spinal fluid were withdrawn by lumbar puncture and 15 c.c. antiserum injected. The fluid showed pus cells and extra- and intracellular diplococci.—12 p. m. Temperature 99.2° .

May 21. Patient conscious and rational; complains of frontal headache. Pupils equal and react; abdominal reflex absent; patellar diminished, plantar present. Kernig's sign marked. Babinski sign absent; ankle clonus absent. Neck rigid and painful on movement. Temperature has risen above 99° .

May 22. Patient's mental condition less good than yesterday. Herpes appearing on lips. Large hyperæmic areas have appeared on abdomen. Temperature during the day has fallen to 99.8° .—10.30 p. m. Under chloroform anæsthesia withdrew 7.5 c.c. of spinal fluid and injected 7.5 c.c. of the antiserum.—12 a. m. Temperature 99.2° .—12.30 a. m. Patient had been resting fairly well when the nurse's attention was attracted by gasps and before a physician could reach her side she died. No autopsy was permitted.

Discussion.—The symptoms of meningitis came on quickly and were severe in type and lumbar puncture and a serum injection were made about 45 hours after their first appearance. Following the puncture and serum injection the temperature remained constantly below 100° and the patient regained consciousness. A second puncture and serum injection were made about 48 hours after the first. About two and a half hours after these the patient suddenly died. No autopsy was obtained and the immediate cause of death was not established. Total amount of antiserum injected, 22.5 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. A. F. Sippy.

CASE VII. H. S. White male, aged 14. Schoolboy.

Present History.—Patient complained of headache on May 21. The next day he vomited and suffered pain in back of head. Temperature, 11 a. m. 101° ; delirious at 5 p. m. Admitted to hospital at 8 p. m.

Physical Examination.—Temperature 104° , pulse 120, respiration 44. Wildly delirious; pupils dilated, do not respond to light. Reflexes absent; Kernig's sign positive; some rigidity of neck.

May 22, 9 p. m. Under chloroform anæsthesia 75 c.c. of opalescent spinal fluid withdrawn and 15 c.c. of antiserum injected. The spinal fluid contained pus cells and extra- and intracellular diplococci. Temperature, 12 a. m., 103° ; pulse 100; respiration 30.

May 23. Patient slept until 2 a. m., after which time he was restless. Temperature: 3 a. m. 102.4°, 9 a. m. 99° 9 p. m. 100° (by rectum).

May 24. Temperature: 12 p. m. 100.6°, 9 p. m. 99.8°. Pulse 60 to 78. Vomited twice; rested fairly well.

May 25. Patient slept fairly well after-part of night; complains of headache. Neck rigid and painful on motion; pupils react to light. Patient conscious and rational. Temperature: 1 a. m. 103°, 9 a. m. 102°. At 10 a. m. 75 c.c. of opalescent spinal fluid withdrawn and 20 c.c. antiserum injected. Microscopical examination of the spinal fluid showed leucocytes and diplococci. Temperature: 12 p. m. 100°, 6 p. m. 99°, and 12 p. m. 98.6° (mouth).

May 26. Temperature normal. Patient slept well after-part of night; conscious and rational; rests quietly; all reflexes present; neck less rigid than earlier in attack.

May 27 to June 1. The temperature has remained normal and his condition satisfactory. Note on June 2 states that the patient is apparently well, the reflexes are normal and the rigidity of the neck has gone.

The final note states that from June 3 to June 8, the day of discharge from hospital, the temperature remained normal and recovery was perfect.

Discussion.—The patient was brought to the hospital during the first day of illness and the diagnosis of meningitis was established by lumbar puncture and the first dose of serum administered in little more than 24 hours after the illness began. The subsequent course of the infection was mild. On the fourth day of illness (May 25) the temperature having risen to 103° F. a second puncture and serum injection were made. Less than twenty-four hours later the temperature had fallen to normal and the general condition of the patient had improved. There was no subsequent rise in the temperature, all the symptoms rapidly subsided, and recovery was complete. Total amount of antiserum injected, 35 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE VIII. R. M. White male, aged 17 years. Electrician with rubber company.

Present Illness.—On May 24 left his work at noon on account of feeling ill. Went home, refused supper, vomited during the evening, and went to bed early on account of headache. Restless during the night. Headache more severe next morning; vomited again, and became unconscious about 10 a. m. Admitted to hospital 1 p. m.

Physical Examination.—Well-developed, muscular man. Pupils slightly contracted; equal; react to light. Neck somewhat rigid and painful on being moved. Knee reflex exaggerated, plantar reflex present, abdominal reflex absent. Kernig's sign present. The patient is very violent and tosses on the bed.

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May 25, 2 p. m. Temperature 105.2°, pulse 142, respiration 20. Under chloroform anæsthesia 15 c.c. of spinal fluid withdrawn and 15 c.c. of antiserum injected. The spinal fluid showed leucocytes and extra- and intracellular diplococci.—6 p. m. Temperature 104.8°, pulse 134.—9 p. m. Temperature 102.4°, pulse 130, respiration 20, cyanosed. Aromatic spirits of ammonia and ether administered hypodermically. Respiration ceased; artificial respiration and oxygen inhalation; respiration continued from 6 to 8 per minute; death at 12.30 a. m.

Discussion.—The preceding case is an example of the fulminating type of epidemic meningitis in which from the first appearance of severe symptoms and death about 24 hours elapsed. Lumbar puncture and serum injection were made about 10 hours before death and were without appreciable effect on the course and termination of the infection. Total amount of antiserum injected, 15 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. George Rankin.

CASE IX. J. A. S. White male, aged 24 years. Mail carrier.

Present Illness.—Until two days before admission to hospital patient has been in good health. He ascribed headache and malaise to exposure to hot sun. June 23 had intense headache, nausea, vomiting and pain in back of neck. About 11.30 p. m. became unconscious. Temperature 103°. Admitted to hospital June 24, 11 a. m.

Physical Examination.—Patient violently delirious; requires to be restrained. Had involuntary bowel and bladder movements. Abdominal and patellar reflexes absent; plantar reflex diminished. Neck rigid; painful on being moved. Kernig's sign present. Pupils equal, moderately dilated, react to light.

June 24, 11 a. m. Temperature 103.8°, pulse 70, respiration 36.—1 p. m. Under local anæsthesia 90 c.c. of spinal fluid removed and 22.5 c.c. antiserum injected. Pus cells and diplococci chiefly within the cells found on examination of the spinal fluid. Temperature: 3 p. m. 100.8°, 12 p. m. 101.4°.

June 25. Patient slept fairly well under an anodyne during the latter part of the night. Note at 8 a. m.: "Patient quiet and semiconscious, responds when spoken to but mutters unintelligibly when attempting to form sentences. Knee jerk not elicited; plantar reflex diminished; abdominal reflex present for first time since admission. Neck rigidly increased." The temperature fluctuated from 99.6° (6 p. m.) to 101.2°.

June 26. Patient restless during night; conscious and rational part of time; neck held less rigidly. The temperature has risen; at 12 p. m. 102.2°, at 12 a. m. 103° (per rectum).

June 27. Restless; extremities cold. 3 a. m. lumbar puncture under chloroform anæsthesia; 45 c.c. of spinal fluid withdrawn and 22.5 c.c. antiserum injected. A chill followed lasting 20 minutes. Herpes appearing on lips. The temperature has fluctuated between 102° and 103° all the day.—9 a. m. "The patient in exceptionally cheerful mood, singing and talking, but entirely rational when spoken to."

June 28. Condition essentially unchanged.

June 29. Temperature at a slightly lower level.

June 30. The note reads: "Condition good; perfectly conscious and rational; reflexes all present; Kernig's sign still marked; neck quite rigid and painful on movement; headache; extensive herpes labialis." The temperature fluctuated between 101° and 103.2°.

July 1. Condition about the same.

July 2, 6 p. m. Temperature: 103.4°, 9 p. m. 103.2°. Under chloroform anaesthesia 60 c.c. spinal fluid, containing diplococci, withdrawn and 30 c.c. of *antisera* injected.—12 a. m. Temperature 101.8°.

July 3. The temperature reached 99° and has run at a lower level than previously.

July 4. The temperature (rectal) has remained below 100° all day. Otherwise the condition is not markedly changed.

July 5. The temperature has fluctuated to-day, having reached 102.2° at 9 a. m., but remaining below 100° for the most part.

July 6. The temperature has risen again. At 6 a. m. it was 103.8°; patient did not rest well; the neck rigidity has lessened; mental condition good. •Kernig's sign still present.—12 p. m. Temperature 103.4°. At 2 p. m. 45 c.c. of spinal fluid were withdrawn and 30 c.c. of *antisera* injected. Stained specimens of the spinal fluid show very few leucocytes containing diplococci and extracellular diplococci.

July 7. Temperature at a lower level, but general condition not essentially changed.

July 8. Patient rested well. The temperature has been below 100° most of the day.

July 9 and 10. Temperature has not risen and condition of patient improved.

July 11 and 12. The temperature has remained normal.

The final note reads: "July 13 to July 27, the date of his discharge from the hospital, the patient continued to improve. When discharged he complained only of not feeling strong. On August 24, when he reported to the hospital, he was well but had not recovered usual strength. On September 8 he had not yet reported for work."

Discussion.—This case of epidemic meningitis ran a moderately severe and protracted course and the symptoms gradually subsided. The particular influence of the spinal punctures and serum injections cannot be defined. The disease terminated favorably and recovery was complete. The conditions under which the serum was employed were favorable to its action since the first injection was made within 48 hours of the onset of severe symptoms. Four injections of the serum were made without the appearance of unpleasant effects. On the other hand the temperature tended to seek a lower level after the injections. The third injection, on the tenth day of illness, was followed by a fall in the temperature

which persisted for four days. When it again rose to 103.4° , on the fourteenth day a fourth injection of the serum was made after which the temperature fell below 100° and soon reached normal where it remained. Convalescence may be said to have begun on the sixteenth day of the illness. Total amount of antiserum injected, 105 cubic centimeters.

City Hospital, Akron, Ohio. Service of Drs. Kobler and Seiler.

CASE X. E. R. White male, aged 20 years.

Present Illness.—The illness began with headache and nausea about noon. After midday meal the patient went to bed and at 3 p. m. was found unconscious and in a violent state. Admitted to hospital the same day (June 30, 1907) at 9.50 p. m.

Physical Examination.—Unconscious and violently excited man moaning constantly. Knee and plantar reflexes exaggerated; Kernig's sign positive; neck slightly rigid; pupils moderately dilated; react to light.

June 30, 10 p. m. Did lumbar puncture under chloroform anæsthesia and withdrew 75 c.c. of spinal fluid and injected 25 c.c. antiserum. Microscopical examination of the fluid showed polynuclear leucocytes and many intra- and extracellular diplococci. Temperature: 10 p. m. 100.2° , 12 a. m. 99° .

July 1, 2 a. m. Convulsion lasting 20 minutes. During the day patient vomited several times. The temperature did not rise above 100° .

July 2. The temperature has been below 100° all day and much of the time has been normal. The note reads: "Condition of patient markedly improved; he is conscious and rational, all the reflexes are present, Kernig's sign is less marked than it was, the neck is still stiff and painful, headache still persists, and the mental condition is good. Considerable nourishment was taken during the day."

July 3. The temperature has remained below 100° and much of the time was normal. The general condition is as on yesterday.

July 4. Patient rested well; he complains less of pain. Temperature normal.

July 5. The note reads: "Patient slept well; general condition good; reflexes normal; Kernig's sign and neck rigidity absent; mentality normal."

The patient continued to have normal temperature and was discharged on July 11 "cured," twelve days after having entered hospital.

Discussion.—The onset of the symptoms in this case was abrupt and severe. From the appearance of the premonitory headache and malaise to the lumbar puncture and serum injection hardly more than twelve hours had elapsed. Following upon the puncture and the injection of serum the symptoms abated rapidly and the patient may be said to have been over the disease within forty-eight hours of its onset. No reasonable doubt can exist regarding the diagnosis in view of the symptoms present and the results of

the bacteriological examination. Total amount of antiserum injected, 25 cubic centimeters.

City Hospital, Akron, Ohio. Service of Drs. Theiss and Sippy.

CASE XI. G. G. White female, aged 17 years. Schoolgirl.

Present illness.—Twenty-four hours before admission to the hospital she began to complain of malaise and headache. During the night she vomited and the symptoms grew gradually worse until 2.30 p. m. when she became unconscious. Admitted to hospital at 7 p. m. June 9.

Physical Examination.—The patient is unconscious and restless. Abdominal reflex absent; patellar and plantar reflexes are diminished; pupils equal; involuntary bowel movements.

June 10, 9 p. m. Temperature 98.4°, pulse 108, respiration 20. Under chloroform anæsthesia 90 c.c. of opalescent spinal fluid withdrawn and 22.5 c.c. of antiserum injected. Microscopical examination of the spinal fluid showed many leucocytes and extra- and intracellular diplococci.—12 a. m. Temperature 98.8°, pulse 124, respiration 20.

June 11. Temperature subnormal most of the day. Patient rested fairly well latter part of night. Twitching of face and mouth; head retracted; rational at times.

June 12. Temperature subnormal. Mental condition greatly improved; abdominal and plantar reflexes present; patellar absent. Kernig's sign marked; neck rigid. Herpes appearing on lips. Complains of no pain unless moved.

June 13. Rested well during night. Mental condition quite good; reflexes all present. Kernig's sign and neck rigidity unchanged. In nurse's absence has gotten out of bed and gone to closet. Temperature normal.

June 14. No change.

June 15. Condition good, all the reflexes normal. Almost no rigidity of the neck; Kernig's sign disappearing.

"From this date the patient continued to improve, and she finally made complete recovery. She left the hospital on June 23, thirteen days after admission, and has reported twice since."

Discussion.—The onset in this case was moderately severe, and the diagnosis of epidemic meningitis was established and a serum injection made within forty-eight hours of the appearance of the premonitory symptoms. The course of the disease was relatively mild, and the temperature tended to remain at the normal point or a little below it although the microscopical examination showed the presence in the spinal fluid of large numbers of *Diplococcus intracellularis*. A second lumbar puncture and serum injection were not made as the symptoms abated rapidly and the patient was convalescent on the fifth day of her illness. Total amount of antiserum injected, 22.5 cubic centimeters.

THE EPIDEMIC OF CEREBRO-SPINAL MENINGITIS AT CASTALIA, OHIO.²

The village of Castalia has a population of about 600 persons. Within the village nine cases of meningitis developed. In the outlying country and within three miles of the village, six cases developed. In the country adjacent to the village of Vickery, which is eight miles from Castalia, three cases developed. Thus a total of eighteen recognized cases of meningitis developed in this region between January and April, 1907. The first case appeared near Vickery in January and the remaining seventeen cases appeared between March 1 and April 2. Eleven of the eighteen cases were in adults over 16 years of age, and seven cases were in children between three and six years of age. Of the affected adults nine died and two recovered and of the affected children three died and four recovered.

In the past thirty years sporadic cases of the disease have appeared occasionally, but at long intervals. No case had been recognized previously for five years.

The distance between cases No. 1 and No. 2 was six miles; between No. 2 and No. 3, three miles, between No. 3 and No. 4 one mile. The first three cases occurred in the country. Case No. 4 appeared in Castalia and was followed in rapid succession by eight other cases. Only two of the cases had been in close personal relation with other persons affected.

At the time that Dr. L. W. Ladd brought the antimeningitis serum to Castalia there had been twelve deaths from epidemic meningitis and three cases were convalescent. He employed the serum on three cases as follows:³

CASE I. B. K. Female, aged 16 years. Patient of Dr. Storey.

Previously healthy girl. Taken ill suddenly March 30, 1907, with headache, vomiting and flushed face. The temperature was 104° F., pulse 124, respirations 48. Coma supervened within 12 hours. The diagnosis was made day after onset, at which time there were opisthotonos and rigidity of the extremities and petechial eruption of neck, trunk and thighs. The patient remained in coma until 12 a. m. March 31, when she became conscious. April 2 again became unconscious. Dr.

² Abstracted from the account of Dr. William Storey, published in the *Ohio State Medical Journal* for June, 1907.

³ The histories of these three cases are taken from Dr. Storey's report and Dr. Ladd's notes.

Ladd first saw the patient at 7 p. m. April 2. At this time she was semi-conscious, the temperature was 103° F., pulse 120, respirations 48. Lumbar puncture was made, about 45 c.c. of very turbid spinal fluid were obtained and 5 c.c. of the antiserum injected. The spinal fluid yielded *Diplococcus intracellularis* on coverslips and in cultures on sheep-serum glucose agar. April 3. Temperature 98.5°, pulse 108. Patient quite rational at times, though when not aroused she was delirious.—3 p. m. Temperature 101°; 11 p. m. 103.5°. Second lumbar puncture made, 30 c.c. turbid fluid withdrawn and 10 c.c. of antiserum injected. The patient remained in a semi-conscious condition for 15 hours when the mental condition cleared; the opisthotonos and rigidity were noted to be much less marked. April 4. Acute bronchitis and broncho-pneumonia developed. The mental condition remained good and the meningeal symptoms were not prominent until April 18, when another puncture was made. About 50 c.c. of turbid fluid were removed and 10 c.c. of antiserum injected. From this time improvement was gradual but steady. April 24, temperature normal; did not rise again. May 3, slight degree of foot-drop on left side. August 31, recovery complete.

Discussion.—The onset in this case was sharp and the course severe. Withdrawal of spinal fluid and injection of antiserum were made on the third day of illness. A favorable response seemed to follow. Two subsequent lumbar punctures and antiserum injections were made on the fourth and fifteenth day of illness. Following the second injection of serum the mental condition cleared and the rigidity of the body was noted as being diminished. The last injection was succeeded by gradual subsidence of all the symptoms and eventual complete recovery. The meningitis was complicated with an intercurrent broncho-pneumonia. Total amount of serum injected, 25 cubic centimeters.

CASE II. F. W. Three years of age. Patient of Dr. Gorsuch.

This child was seen by Dr. Ladd twelve days after the beginning of the symptoms. The onset, consisting of vomiting, convulsions, headache, opisthotonos and great irritability, was sudden. Temperature at onset 104°, pulse 135, respirations 40. The patient had improved somewhat, although there was still great irritability and marked opisthotonos; temperature from 101° to 102.5°. April 3, marked opisthotonos and Kernig's sign; temperature 102°. Lumbar puncture yielded 30 c.c. of moderately turbid fluid. 5 c.c. antiserum injected. That night, for the first time during the illness, the parents were not aroused. April 4, child less irritable; opisthotonos and rigidity less marked; food taken better. The temperature reached normal and remained so afterwards. April 28, child up and about. August 31, child well.

Discussion.—The abrupt change in the condition in this case was evidently associated with the withdrawal of spinal fluid and the

injection of the antiserum, but the part each played cannot be estimated separately. Total amount of serum injected, 5 cubic centimeters.

CASE III. J. B. Aged 23 years. Patient of Dr. Bowman. Employed on railroad.

Large man of excellent physique. On April 1 went to Freemont, Ohio, to a hotel. Asked to be called early April 2. The door of his room had to be forced open; the patient was found unconscious. He was removed to his home in Vickery and his physician called. On April 4 Dr. Bowman was convinced of the diagnosis of cerebro-spinal meningitis and sent to Castalia for Dr. Ladd.

Note by Dr. Ladd: Man of powerful build; comatose and restless; thrashing about the bed at times. Marked opisthotonos; purulent conjunctivitis; Kernig's sign present; petechial eruption and larger hæmorrhagic areas on body. Nose-bleed requiring packing to control. Temperature 103.5°, pulse 120, respirations 48 and stertorous. Lumbar puncture; 90 c.c. of turbid fluid removed; 10 c.c. of antiserum injected. Regarded the condition as hopeless. April 5, in the morning patient still unconscious. Pulse was lower, temperature normal, respirations normal. In afternoon second puncture. 45 c.c. of less turbid fluid removed and 10 c.c. of antiserum injected. April 6, 10 c.c. of antiserum injected. Patient still unconscious. April 7, patient coming out of unconscious state. The progress was fluctuating and the patient was not without fever for 30 days. The improvement was gradual and the restoration complete. The patient returned to his work.

Discussion.—The case was one of abrupt and violent onset and of gradual subsidence of symptoms. The first puncture and injection of the serum were made on the fourth day of illness and were followed rapidly by improvement in the temperature, pulse and respirations. Two further injections of serum were given. The recovery was slow and complete. Total amount of serum injected, 30 cubic centimeters.

The epidemic of Castalia embraced 18 cases of meningitis of which 12 died and 6 recovered. Three of the latter recovered without lumbar puncture being performed and without the antiserum being injected. The three cases which were injected with the serum recovered. As these were the first cases in series to be injected with the serum the doses employed were smaller than the ones used subsequently.

The first case was injected with serum about 72 hours after the onset of the disease. Improvement was gradual and the symptoms subsided by lysis, hence it is not possible to assign certainly the

precise value of the serum injections. The second case, which was already in its second week of the disease, seems to offer more certain proof of the value of the puncture and serum injection. The disease terminated abruptly after the injection of 5 cubic centimeters of the serum and recovery was rapid and complete.

The third case was regarded as hopeless. Lumbar puncture and injection of antiserum having been made, the patient's condition changed quickly for the better, but the final recovery was fluctuating and gradual. The precise value of the serum injections must therefore remain doubtful.

EMPLOYMENT OF THE SERUM BY DR. L. W. LADD, OF CLEVELAND.

Dr. Ladd treated in all sixteen cases of epidemic meningitis in which the diplococcus was found with the antiserum in Castalia and Cleveland. He has kindly supplied us with the notes of his cases which will be presented in this place. Since three of the cases treated by Dr. Ladd were at Castalia the histories relating to them are given under the local epidemic of which they formed a part. We will first present Dr. Ladd's own analysis of the sixteen cases, then an analysis of our own, after which the case-histories of each to which we have added a brief discussion will be given.

"The sixteen cases consisted of 11 males and 5 females. Five patients were over 16 years, eight under 5 years and three between 5 and 8 years of age. When first seen thirteen of the sixteen cases were in coma, one was semi-comatose and two were conscious. Five patients were seen 24 hours or slightly earlier after the onset of symptoms. All these cases recovered completely. One patient was seen 48 hours after the onset. The condition was desperate. The patient died. Four patients were seen approximately 72 hours after the onset. They recovered. Two of these cases recovered completely, one developed foot-drop and recovered subsequently, one had impaired hearing when last seen. Three patients were seen approximately 96 hours after the onset. They died. Two of these cases showed a temporary improvement following lumbar puncture and serum injections. Two patients were seen two weeks after the onset, one recovered, the other died of chronic hydro-

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cephalus. One patient was seen one month after onset and died of chronic hydrocephalus."

The percentage of recoveries among the sixteen cases was 68.75 and the deaths 31.75. Taking the ten cases treated with serum within 72 hours of the onset of symptoms, nine recovered and one died. The percentage of recoveries in this series is 90. Two of the five cases ending fatally were injected with the serum 96 hours or thereabouts after the onset and two cases several weeks after the beginning of the disease.

Case	I, 1st injection on 3d day; total serum injection 25 c.c.; recovery by lysis.					
" II,	"	" 12th	"	"	"	5 c.c.; " crisis.
" III,	"	" 4th	"	"	"	30 c.c.; " lysis.
" IV,	"	" 5th	"	"	"	15 c.c.; died.
" V,	"	" 2d	"	"	"	35 c.c.; recovery by crisis.
" VI,	"	" 1st	"	"	"	15 c.c.; " "
" VII,	"	" 3d	"	"	"	20 c.c.; " "
" VIII,	"	" 3d	"	"	"	45 c.c.; " lysis.
" IX,	"	" 2d	"	"	"	35 c.c.; died.
" X,	"	" 1st	"	"	"	15 c.c.; recovery by crisis.
" XI,	"	" 20th	"	"	"	25 c.c.; died.
" XII,	"	" 4th	"	"	"	46 c.c.; " "
" XIII,	"	" 1st	"	"	"	45 c.c.; recovery by crisis.
" XIV,	"	" 14th	"	"	"	28 c.c.; " lysis.
" XV,	"	" 3d	"	"	"	53 c.c.; " "
" XVI,	"	" 4th	"	"	"	41 c.c.; died.

Scrutinizing this table closely we find that of the 11 patients who recovered the disease terminated, after serum injection, by lysis in five and by crisis in six cases. It is somewhat noteworthy to find that a case of meningitis which has lasted twelve days without intermission of symptoms should terminate abruptly after a lumbar puncture and serum injection. But of the several important cases terminating abruptly by crises where the serum injections were made on the first to third day, the most significant is case XIII in which the injection was made about two hours after the first appearance of the meningeal symptoms with the result of immediately arresting the progress of the disease.

A second tabulation dealing with the cases in children under five years of age, owing to the high mortality of epidemic meningitis in infants will be given.

Case	II, Age	3 years; recovered; 1st injection on 4th day of illness.					
"	V, "	3	"	"	"	2d	" "
"	VI, "	2	"	"	"	3d	" "
"	X, "	2	"	"	"	1st	" "
"	XI, "	1½	"	died	"	20th	" "
"	XII, "	2	"	"	"	4th	" "
"	XIV, "	1½	"	recovered	"	14th	" "
"	XVI, " under 5	"	"	died	"	4th	" "

Of the eight cases of this tabulation five recovered and three died. The three deaths occurred in children who received the serum for the first time on the twentieth and the fourth day of the illness respectively. On the other hand two children who were first injected on the fourth day, and one child injected on the fourteenth day recovered. There is no proof that the course of the disease in the last case was essentially influenced by the antiserum.

CASE I. B. K. Castalia epidemic. Recovered.

CASE II. F. W. Castalia epidemic. Recovered.

CASE III. J. B. Castalia epidemic. Recovered.

CASE IV. H. White male, aged 17 years. Barberton, Ohio.

The patient was employed at the Goodrich Rubber Co.'s factory at Akron. May 2, in the evening, he complained of fever and headache. He was seen by Dr. Lahmers May 3. At that time there were diarrhoea and vomiting, but no fever. The same evening meningeal symptoms and coma came on. May 6 Dr. Ladd saw the patient. There were extreme opisthotonos—the head almost touched the scapulae—general rigidity, Kernig's sign, petechiae over body, and purulent conjunctivitis. Temperature 103.5°, pulse 85, respirations 30. Lumbar puncture yielded two or three drops of thick pus. *15 c.c. of antiserum were injected.* No beneficial effect was noted. Died May 7.

Discussion.—The character of the exudate in this case and the general condition of the patient five days after the beginning of the infection, probably operated against any beneficial effects resulting from the serum. Total amount of serum injected, 15 cubic centimeters.

CASE V. W. F. Male, aged 3 years. Bohemian.

On March 31 complained of headache in afternoon. April 1, severe headache; vomited. From 1 until 5 p. m. five convulsions, each lasting five minutes.—5 p. m. Coma, opisthotonos and Kernig's sign present; abdominal reflex absent; small petechiae over body.—9 p. m. Lumbar puncture: 30 c.c. turbid spinal fluid removed and 10 c.c. of antiserum injected. Temperature 99.4°, pulse 125, respirations 30.

April 2. Patient improved. Temperature and respiration unchanged, opisthotonos less marked, abdominal reflex present. Child conscious and rational; took nourishment.

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April 6. Until to-day the child has done well. Temperature suddenly rose to 103.5°, pulse 116.

April 7. Lumbar puncture done in morning and 30 c.c. turbid fluid removed and 15 c.c. of serum injected. The child was delirious before the puncture.

April 8. Temperature 99.6°, pulse 120.

April 22. Child did well until to-day, when the temperature rose to 103.2°; pulse 132. Vomiting and meningeal symptoms present. Lumbar puncture removed 30 c.c. of fluid; 10 c.c. of antiserum injected.

April 24. Temperature normal. From this date on the recovery was uneventful and finally was complete.

Discussion.—The first lumbar puncture and serum injection were made within 48 hours of the onset of symptoms. The disease seemed to have been promptly arrested by the injection. Subsequently two relapses occurred, one on the sixth and the other on the twenty-second day, which were as abruptly arrested as the first symptoms by the lumbar puncture and serum injection. Recovery was complete. Total amounts of serum injected, 35 cubic centimeters.

CASE VI. Baby N. Female, aged 2 years. Italian.

Child lived in the poor district of Cleveland. Dr. Steuer, the attending physician, diagnosed the case as meningitis and called Dr. Ladd—22 hours after the onset of symptoms. Child had marked opisthotonos, lateral nystagmus, Kernig's and MacEwen's signs and muscular rigidity. She was completely comatose. Temperature 102.5°, pulse 140, respirations 40. Lumbar puncture: 50 c.c. of turbid fluid removed and 15 c.c. of antiserum injected. Morning of next day, temperature, pulse and respirations normal; child dull and listless but conscious. Kernig's sign and opisthotonos less marked. Parents refused second puncture and injection of serum. The meningeal symptoms quickly subsided and recovery was complete. A few days later whooping cough developed, from which recovery was finally made.

Discussion.—The essential facts in this case are the sudden onset of severe symptoms of meningitis in an infant of two years and their abrupt arrest and permanent and rapid dissipation after lumbar puncture and serum injection performed in the first twenty-four hours of the disease. Total amount of serum injected, 15 cubic centimeters.

CASE VII. X. Female, aged 11 years. American.

May 1. Patient seized with severe headache, vomiting, stiffness of neck. 12 hours later unconscious. Opisthotonos marked; Kernig's sign present; lateral nystagmus; petechial eruption. May 4 Dr. Ladd saw patient and did lumbar

puncture. 30 c.c. of turbid fluid removed and 10 c.c. of *antiserum injected* (this within 72 hours of the onset of the symptoms). Temperature 103.6°.

May 2. Temperature and pulse normal; patient answered questions rationally though lapsed into semi-consciousness when left alone.

May 6. Improved.

May 7. Temperature rose and meningeal symptoms became prominent. Lumbar puncture repeated; 10 c.c. *antiserum injected*. Temperature fell to normal. Complete recovery.

Discussion.—The prompt amelioration of the severe symptoms by the first lumbar puncture and serum injection and the equally prompt suppression by these means of what threatened to be a relapse of the disease, are striking incidents of this case. Total amount of serum injected, 20 cubic centimeters.

CASE VIII. W. H. White male, aged 21 years. Hudson, Ohio. Farmer.

May 12. Had a chill in evening. May 13. Unable to work.—8 a. m. Had a second chill and severe headache. In a few minutes was delirious and soon became unconscious. Dr. Ladd saw the patient May 14. There were present marked opisthotonos, Kernig's sign, general muscular rigidity and petechial eruption. (It developed that two weeks before the patient's mother had nursed a fatal case of epidemic meningitis.) Lumbar puncture was attempted four times but only a few drops of bloody serum were obtained. This fluid showed doubtful diplococci. The culture was negative. Clinically there was no doubt of the diagnosis. In view of the fact that no spinal fluid was obtained, 5 c.c. of *antiserum* were injected into the canal and 10 c.c. *antiscrum under the skin*. Temperature 103.5°.

May 15. Patient improved; rational at times, opisthotonos, muscular rigidity and Kernig's sign less marked. 15 c.c. *antiserum injected subcutaneously*. The temperature ranged from 100° to 103°, when, on the twelfth day, it fell to normal and remained there. On the seventh day of illness another dose of 15 c.c. of the *antiscrum* was given subcutaneously. A marked erythematous rash appeared on the fifteenth day. Recovery was complete.

Discussion.—The absence of bacteriological confirmation of the diagnosis in this case is an evident weakness, but there would appear to be little doubt of the nature of the disease. What is noteworthy is the subcutaneous employment of the antiserum. The symptoms gradually subsided by lysis and hence the special influence of the serum cannot be defined with certainty. Total amount of serum injected, 45 cubic centimeters.

CASE IX. C. G. White female, aged 6 years.

The patient was one of three children in one family afflicted with meningitis. June 30, at 4 a. m., was taken with headache and vomiting. July 1, at 10 a. m.,

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had a convulsion. She was brought comatose to the City Hospital. There were marked Kernig's sign and opisthotonos. Extensive petechiæ and herpes oris. July 2, 8 p. m., lumbar puncture yielded 5 c.c. very turbid fluid. 10 c.c. of antiserum were injected.

July 3, 10.30 a. m. Lumbar puncture done and 15 c.c. very turbid fluid removed. 10 c.c. antiserum injected intraspinally and 5 c.c. subcutaneously.

July 4. Right-sided hemiplegia developed. Patient's condition still very bad.

July 5, 11 a. m. Lumbar puncture and administration of 10 c.c. antiserum.—7 p. m. Condition very bad. Temperature 107.4°. Death. Consciousness never regained.

Discussion.—The onset of symptoms was in this case rapid and severe. The first lumbar puncture and serum injection were made about 64 hours after the first symptoms were noted, and while the patient was in an unconscious state. Two later injections of the serum were given, but no beneficial effect followed any of the injections. Total amount of serum injected, 35 cubic centimeters.

CASE X. J. C. White male, aged 2 years.

This is the second child having meningitis in this family. July 1, patient put to bed in good health. Later in the evening he was found restless and feverish. The physician made a diagnosis of meningitis. Admitted to City Hospital July 2, 9 a. m.

July 2. On admission marked opisthotonos, Kernig's sign and general petechial eruption were noted. Child comatose. At 4 p. m., approximately 20 hours after onset of symptoms, lumbar puncture performed and 50 c.c. of turbid fluid removed; 10 c.c. of antiserum injected into spinal canal and 5 c.c. under the skin. Temperature at this time was 104°, pulse 140, respirations 62.—7 p. m. Temperature 105.6°, pulse 148, respirations 78.

July 3. This morning temperature 100°, pulse 128, respirations 40. Child conscious and irritable.

July 4, 10.30 a. m. Lumbar puncture negative (probably unsuccessful). The patient's condition quickly improved and, except for a slight rise of temperature to 102.4° at 12 a. m. July 5, the temperature, pulse and respirations remained normal.

July 17. No complications have developed. Child discharged cured.

Discussion.—The onset of symptoms in this case was sudden and severe. Twenty hours after the symptoms appeared lumbar puncture and serum injection were performed. Within twenty-four hours of these operations the condition had changed abruptly for the better and the disease had terminated by crisis. Recovery was uninterrupted. Total amount of serum injected, 15 cubic centimeters.

CASE XI. P. G. White male, aged 18 months. Austrian.

March 12. Admitted to Lakeside Hospital. Until March 4 the child had been well since birth. On that day was fretful and feverish, coughed and had diarrhoea. March 7, right elbow swollen and painful; neck stiff; macular eruption over body. On admission to Hospital there were irritability, large head retracted to right side, slight strabismus, pupils equal and active to light, slight swelling about flexed and rigid right elbow.

March 23. Temperature 105° . Lumbar puncture: 25 c.c. slightly turbid spinal fluid removed. On microscopical examination a small number of leucocytes containing diplococci of typical intracellularis appearance seen. No change in condition as result of puncture. Lumbar puncture every third or fourth day as routine. Temperature ranged from 98° (a. m.) to 104° (p. m.). No marked change in the physical condition.

April 12. Lumbar puncture and *intraspinal injection of 5 c.c. serum*.

April 14. Temperature did not rise above 100.5° , which was the lowest maximum temperature since admittance.

April 16, a. m. Temperature normal. *7 c.c. antiserum injected*. Temperature normal until April 23, when it rose suddenly to 104° , pulse 140, respiration 40. Symptoms of chronic hydrocephalus developed. Several additional punctures and injections of serum were made without beneficial effect. Death on July 30.

Discussion.—The first lumbar puncture was made on the twentieth day and the first serum injection was given on approximately the fortieth day of illness. Following the latter the symptoms diminished and there seemed decided improvement lasting eleven days. The symptoms reappeared and death resulted from chronic hydrocephalus. Total amount of serum injected, more than 25 cubic centimeters.

CASE XII. F. P. White male, aged 3 years.

Onset sudden on May 18 with headache, vomiting and temperature to 106.5° . Bright red pin-point rash over body. May 20, first convulsion noticed. Opisthotonos pronounced.

May 22 (about 96 hours after onset). Lumbar puncture and 45 c.c. of very turbid fluid withdrawn. Two hours later second puncture, 20 c.c. fluid removed and *15 c.c. antiserum injected*. Temperature 103° .

May 23. Patient brought to Lakeside Hospital. Temperature 101° , pulse 140, respirations 40. Conscious, very irritable, opisthotonos and Kernig's sign moderately marked (as on 22d, when seen by Dr. Ladd). Petechial eruption present.—4 p. m. Lumbar puncture: 50 c.c. turbid fluid withdrawn and *15 c.c. antiserum injected*.—12 a. m. Temperature 101.6° .

May 24. Patient drowsy; neck rigidity increased. 8 a. m. Temperature 102° .—3 p. m. Lumbar puncture: 40 c.c. turbid fluid removed and *16 c.c. serum injected*. Temperature 103.5° , pulse 135.—4 p. m. Chill, marked cyanosis, pulse feeble, respirations poor.—4 p. m. Temperature 106° . After tub bath temperature 104° .—9 p. m. Chill, cyanosis, temperature 106.5° . Death at 1.25 a. m., May 25.

Discussion.—The first lumbar puncture and serum injection were made 96 hours after the onset of symptoms, and on the next two successive days the punctures and injections were repeated. The disease progressed rapidly and continuously and ended fatally approximately six days after the onset. The question arises whether the sudden change for worse on May 24 bore any relation to the puncture and injection of serum one hour previously. There are no data at hand to use in answering this question. In other cases in which daily injections of serum were made severe symptoms did not appear. Total amount of serum injected, 46 cubic centimeters.

CASE XIII. O. C. White female, aged 7 years. Sister of F. C., Case XII.

Until May 21 child well. Symptoms began with fever (to 106°), severe headache, thirst and erythematous eruption over arms and legs. May 22, 2 p. m., child seen by Dr. Ladd.

May 22. Child conscious. No Kernig's sign. No opisthotonos, rigidity of neck or extremities. A bright erythematous eruption over chest and extremities. During the two hours which were employed in the examination of the fluid withdrawn from brother, marked meningeal symptoms developed, convulsions and opisthotonos and coma set in. Lumbar puncture was done, 30 c.c. of turbid fluid were withdrawn, and 15 c.c. of antiserum injected intraspinally. The patient spent a quiet night and was brought to the Lakeside Hospital May 23.

May 23, a. m. Temperature 100° , pulse 120, respirations 35. Considerable restlessness. Moderate opisthotonos and rigidity of extremities. Unconscious. Kernig's sign present.—4 p. m. Lumbar puncture: 25 c.c. serum under moderate pressure removed and 15 c.c. serum injected.

May 24. Condition greatly improved. Opisthotonos almost gone. Kernig's sign diminished, child less irritable and conscious though dull. Temperature normal, except for rise to 101.5° following puncture and injection of 15 c.c. of antiserum. Only a few drops of turbid fluid obtained.

May 25. Temperature normal; mind clear; meningeal symptoms absent. Recovery uninterrupted. The disease in this case terminated in four days from its beginning.

Discussion.—The symptoms in this case set in abruptly and with much intensity. Within the first 24 hours of illness the diagnosis was established by lumbar puncture and the first serum injection made. What is especially important in this case is the fact that the puncture and serum injection were made in less than two hours after the appearance of symptoms due to meningeal irritation. The disease appears to have been arrested by the first puncture and

injection and, although two subsequent injections of serum were made, the symptoms disappeared rapidly after the first injection and the patient was well on the fourth day from the onset of the illness. Total amount of serum injected, 45 cubic centimeters.

CASE XIV. L. S. White male, aged 1 year and 7 months. Pole.

May 25. Admitted to Lakeside Hospital. Two weeks before the child fell while playing and lay for a few moments with head retracted. In a little while fell again in convulsion. On undressing him the mother noticed an eruption on the body. During the next five days the child had four convulsions; has been in bed since with head retracted and squint. On admission to hospital there were noted emaciation, marked opisthotonos and internal squint. Kernig's sign present. Lumbar puncture gave 25 c.c. turbid fluid. Temperature 101°, pulse 165, respirations 45. *5 c.c. antiserum injected*. Temperature normal during next four days.

May 28. Temperature 101.5°. Lumbar puncture gave 15 c.c. clearer fluid. *6 c.c. antiserum injected*. From the first puncture and injection the opisthotonos diminished and the mental condition brightened. Until June 1 temperature normal.

June 1, 12 a. m. Temperature 103.5°; next morning normal. Child gaining steadily in weight.

June 12. Temperature 103°. Lumbar puncture yielded 25 c.c. of clear spinal fluid in which a small number of diplococci were still present. *7 c.c. antiserum injected*. Until June 25 steady improvement.

June 25. Temperature 102°. *5 c.c. of serum given under the skin*. Temperature returned to normal but on June 27 again rose to 102°. *5 c.c. serum given subcutaneously*. No further rise occurred. Discharged cured.

Discussion.—This case is an example of sub-acute epidemic meningitis in which the symptoms rapidly ameliorated after lumbar punctures and serum injections began two weeks after onset of the disease. The only point that is especially noteworthy is the rapid clearing of the spinal fluid after the first puncture and serum injection. No statement of the precise part played by the serum can be made. Total amount of serum injected, 28 cubic centimeters.

CASE XV. C. D. White male, aged 8 years. German.

The patient was well until the night of June 13. Onset of disease was with irritability, severe headache and vomiting. June 16, diagnosis of meningitis made. There were present marked opisthotonos, muscular rigidity of extremities and petechial eruption. Patient comatose, respirations stertorous.

June 16, 1 p. m. Temperature 103°. Lumbar puncture gave 45 c.c. of turbid fluid; *10 c.c. antiserum injected*.—8 p. m. 45 c.c. spinal fluid removed and *5 c.c. antiserum injected*. The symptoms ameliorated somewhat during the day.

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June 17. Temperature and pulse normal. Toward evening patient can be made to respond to loud questions but answers are unintelligible.

June 18, a. m. Temperature 104° ; restless.—9.50 p. m. Lumbar puncture: 40 c.c. of turbid fluid withdrawn and 15 c.c. of *antiserum injected*.

June 19, 12 a. m. Patient quiet, temperature normal. Mental condition clearer, temperature not above 101.5° for two days.

June 21. Restlessness; temperature 104° . Lumbar puncture: no fluid obtained, 5 c.c. *antiserum injected under the skin*.

June 22. Temperature normal.

June 25. 5 c.c. *serum subcutaneously*.

June 26, 12 a. m. Temperature 103° .

June 27, 8 a. m. 5 c.c. *serum subcutaneously*. Temperature remained below 102° until June 30, when it rose suddenly to 104° .

June 30 to July 8. Up and down temperature.

July 8. Owing to increased irritability and symptoms of meningeal irritation lumbar puncture made and 35 c.c. of nearly clear fluid withdrawn.

July 10. After two days of nearly normal temperature there was a rise to 105.8° . Lumbar puncture made and 8 c.c. *antiserum injected*.

July 11. Temperature normal. No further rise from this time. Patient discharged on July 29 well except for slight impairment of hearing.

Discussion.—The first puncture and serum injection were made on the third day of the disease and were followed by improvement in the patient's condition. The course of the illness was fluctuating and the symptoms subsided gradually and with occasional exacerbations. The lumbar punctures and serum injections, made during the access of the symptoms, appeared to control the fever and reduce the restlessness, while the subcutaneous injections of the serum would appear to have been less effective in this respect. Recovery was incomplete as impairment in hearing remained. Total amount of serum injected, 53 cubic centimeters.

CASE XVI. I. L. White, aged under 5 years. Hebrew.

June 18. Until to-day well. Complains of headache. June 19. Severe headache, vomiting, opisthotonos, petechial spots on body. In the evening unconscious. June 22. Admitted to Hospital (approximately 96 hours after onset). Symptoms unchanged except for exophthalmos on the right side and a lateral nystagmus. Lumbar puncture yielded 30 c.c. of very turbid fluid. 6 c.c. of *antiserum injected*. Temperature fell from 104° to 100° in four hours; muscles less rigid; mind clearer.

June 23, a. m. Spinal puncture: 30 c.c. fluid removed and 5 c.c. *serum injected*.

June 24, 4 p. m. Spinal puncture: 35 c.c. fluid removed and 5 c.c. *serum injected*.

June 25. 5 c.c. *serum given subcutaneously*. Following this the temperature which had ranged from 104.5° to 100° fell to normal.

June 27, 8 a. m. Temperature rose to 103°. Lumbar puncture, 2.5 c.c. reddish serum obtained and 5 c.c. serum injected. Until July 9 child seemed to improve. The opisthotonos had diminished; temperature slightly elevated, sometimes reaching 104°.

July 9. Lumbar puncture: 45 c.c. of fluid removed and 5 c.c. of antiserum injected.

July 13. 37 c.c. fluid withdrawn by puncture and 10 c.c. serum injected. The temperature remained fluctuant; symptoms of increasing hydrocephalus developed and punctures and serum injections were repeated, but death took place on July 31, 1907.

Discussion.—This child was first injected with serum on the fourth day of the illness after which the acute symptoms ameliorated somewhat. The progress of the disease was not arrested by the several punctures and serum injections and chronic hydrocephalus and death resulted. Total amount of serum injected exceeded 41 cubic centimeters.

THE CASES OF EPIDEMIC MENINGITIS AT PHILADELPHIA.

The cases of meningitis at Philadelphia followed on the heels of the large epidemic at New York and probably constituted part of that epidemic. The report of the use of the antiserum at the Pennsylvania Hospital, supplied by Dr. Longcope, embraces five cases of the disease. Of these, four recovered and one died. We will tabulate these cases according to the period of the first injection of the serum, the total amount of antiserum employed in each case, and the mode of termination of the disease.

Case	I,	1st injection on 4th day; total serum injection 15 c.c.; recovery by crisis.
"	II,	" " 3d " " " 25 c.c.; " lysis.
"	III,	" " 4th " " " 45 c.c.; " crisis.
"	IV,	" " 11th " " " 25 c.c.; death.
"	V,	" " 10th " " " 15 c.c.; recovery by lysis.

Pennsylvania Hospital, Philadelphia. Service of Dr. M. J. Lewis.

CASE I. J. J. Negro male, aged 23 years. Stevedore.

Present Illness.—On the morning of July 2, 1907, the patient experienced headache, indigestion, weakness and exhaustion and pain in the back. During the afternoon the headache increased in severity, and a sense of malaise and illness was felt. He was brought to the receiving ward by the patrol service and remained over night. The next morning, as he felt better, he left the hospital and went to the wharf and lay down. Soon afterwards he became nauseated and vomited freely. The pain in the head returned with increased severity and there were pain and stiffness of the back of the neck, chilly and feverish sensations and

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general pain of the body. That night (July 3) the patient went home and to bed and in spite of being very ill he went to work the next day. Later he was found unconscious on the wharf and was brought to the hospital (July 4). The note on admission states that the patient is unconscious, the neck is rigid, the urine is voided involuntarily, the temperature is 101° F., the pulse 80, and the respirations 26.

Physical Examination.—Large, robust negro lying unconscious but restless; irrational and somewhat noisy. Skin hot and dry; no rash; pupils react equally to light; slight degree of nystagmus and strabismus; conjunctivæ injected; tongue moist and heavily coated; breath offensive; moderate degree of rigidity; Kernig's sign present; patellar reflexes practically absent; sensations appear to be normal. (Nothing abnormal was discovered in heart, lungs and abdomen.) The urine showed a faint trace of albumen and a small number of hyaline and granular casts and leucocytes, but no sugar.

July 5. The alterations since yesterday are increased rigidity of neck and more marked Kernig's sign. Leucocyte count 15,800.—1 p. m. Lumbar puncture: 20 c.c. of very turbid fluid showing many *Diplococcus intracellularis* and pus cells obtained.—5 p. m. Second puncture: 65 c.c. fluid evacuated and 15 c.c. *antimeningitis serum injected*. Temperature 100° F.—6 p. m. Temperature 102.2°, respirations increased, some degree of relaxation, patient quieter and less delirious apparently.—6.30 p. m. Temperature 101°, pulse 96, respirations 32.—9 p. m. Temperature 99.3°, pulse and respiration improved, patient more relaxed and has been perspiring, pupils react to light.—10.40 p. m. Partly rational and can be aroused sufficiently to give name, address and occupation; relaxation still more marked; headache is complained of.

July 6. Condition improved. Temperature 98.3°, pulse 60, respiration 20. Neck slightly rigid only, Kernig's sign greatly diminished; pupils equal and react; conjunctivæ clear; nystagmus and strabismus diminished; patient quiet and rational but is drowsy and somewhat stuporous. The next note reads: "This evening the temperature has gone up again; otherwise the general condition is much improved."

July 7. Temperature normal; condition improved; symptoms abating; headache, strabismus and muscular rigidity less marked; some diplopia present. Bowels moved freely.

July 8. Temperature 100°, otherwise no important change.

July 9. The note reads: "Temperature, pulse and respiration are normal. The patient is much brighter, headache has almost disappeared, the body is almost perfectly relaxed, patellar reflexes are normal, there is only a faint suggestion of Kernig's sign, the diplopia is less marked, but there is strabismus and paralysis of the left external rectus. The bowels have moved and the patient complains of hunger."

July 10. The urine contains a trace of albumen but no casts. Leucocytes 7,160.

July 11. Condition good, no discoverable rigidity, no headache, patient bright and hungry, tongue clean, temperature normal. Allowed to sit up.

July 12. All the symptoms except paralysis of external rectus and slight degree of diplopia have subsided; a suggestion of Kernig's sign remains. The diet is increased and the patient is allowed out of bed for a few minutes.

July 15. About ward.

July 18. Diplopia and Kernig's sign seem to be disappearing; still some paralysis of external rectus.

July 21. Feels well; can read without difficulty. No headache.

July 24. Improvement continues, paralysis of external rectus diminishing; all other symptoms have subsided.

July 26. "Discharged cured."

Discussion.—The first symptoms of meningitis appeared on July 2, and it is probable that lumbar punctures made at that time might have developed the nature of the disease. The symptoms increased in severity progressively until July 4 when they became marked. In view of the almost unavoidable uncertainty attaching to a determination of the onset of the disease it is safe to count July 3 to 4 as the first twenty-four hour period of its actual existence. Hence the serum was injected about 48 hours after the development of unmistakable symptoms. The symptoms abated rapidly after the second lumbar puncture and the injection of serum, and the patient experienced no relapses but improved progressively and made a complete recovery. Total amount of serum injected, 15 cubic centimeters.

Pennsylvania Hospital, Philadelphia. Service of Dr. J. C. Wilson.

CASE II. R. B. White male, aged 17 years. Clerk.

Present Illness.—On June 27, 1907, the patient was brought to the hospital in ambulance. He complained of malaise, severe headache, vomiting and constipation. The illness began on June 25 with malaise and headache. The same night the symptoms became suddenly much worse: headache, severe aches and pains of body, nausea and vomiting, chilly and feverish sensations, and alternating delirious and lucid periods until morning. The symptoms continued the next day and rigidity of neck was complained of. On admission temperature 100°, pulse 100, respirations 28.

Physical Examination.—Well-developed, robust-looking boy. Lies quietly in bed, although he tends to be restless and irritable; is rational but stuporous. Skin dry and hot; no rash; no herpes; face flushed; pupils slightly dilated and equal; react; conjunctivæ clear; tongue heavily coated and dry; head slightly retracted and rigid and painful. Kernig's sign marked; suggestion of Babinski reflex; patellar reflexes practically absent; some cutaneous hyperæsthesia; leucocytes 16,500 (nothing abnormal was discovered in heart, lungs and abdomen).

June 28. Condition essentially unchanged. The head is held more rigidly possibly and much headache is complained of.

June 29. Herpes appeared on lips; lumbar puncture at 3 p. m. and moderately turbid fluid obtained. 10 c.c. of antimenigitis serum injected slowly into canal. No microorganisms found in the spinal fluid. One hour after the injection the temperature dropped from 100.6° to normal, the pulse became softer and ranged

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from 74 to 64; respirations unchanged. Rigidity unchanged. In the evening temperature rose to 99.6°; patient very restless; morphia administered hypodermically.

June 30. Temperature 100.4°, dropping later to 98.4°; patient somewhat more comfortable; pulse 72. Evening: patient seems better, less pain and rigidity of head; has been sleeping or dozing much of the time; takes nourishment; bowels freely opened. Leucocytes 15,800.

July 1, 4.30 p. m. Lumbar puncture and 80 c.c. fluid removed; 15 c.c. *antimeningitis serum* injected.—7 p. m. Temperature has risen from 98.5° to 101.5°. Patient perspired freely. No microorganisms found on cover-slips but many leucocytes. One colony of Gram-negative diplococcus grew on calf-serum agar.

July 2. Kernig's sign present; pain in back and legs; no headache; rigidity of head diminished. Leucocytes 10,550.

July 3. Has been very comfortable; no morphia given since July 1; no headache; rigidity of neck much lessened so that the head can be brought pretty well forward. Kernig's sign much diminished; no bodily discomfort; temperature normal or slightly subnormal.

July 4. Quite comfortable; hungry; given ice cream and orange juice. Kernig's sign diminishing.

July 6. Slight evidence of Kernig's sign; comfortable; on soft diet.

July 9. Up on bed rest.

July 16. Stiffness and pain in neck entirely and Kernig's sign practically gone. Temperature subnormal. Discharged.

Discussion.—The disease in this case began, probably, on June 25, the symptoms growing severe within 24 hours of their first appearance. The diagnosis of epidemic meningitis is rendered probable but not certain by the bacteriological examination. Lumbar puncture was performed and anti-meningitis serum injected twice. The first injection was made on approximately the third day, and the second on the sixth day of the disease. The recovery of the patient was progressive and complete without relapses.

Pennsylvania Hospital, Philadelphia. Service of Dr. Newlin.

CASE III. A. G. White female, aged 15 years. Canvas weaver.

Present History.—Went to work on September 6, but came home at noon on account of pain in head and back of neck. September 7 and 8 vomited several times. Since 6th inst. has been delirious and feverish and crying out. Admitted to hospital September 8.

Physical Examination.—Well-developed young girl lying in bed with chin thrown up and neck held stiffly; eyes injected; pupils equal, react; tongue heavily coated, dry; herpes about mouth; crying out; no rash. Kernig's sign well marked; knee jerks present, no ankle clonus. Nothing abnormal discovered in heart, lungs and abdomen.

September 9. Lumbar puncture yielded 3 c.c. of very turbid fluid containing *diplococcus intracellularis*. Leucocytes 23,000.

September 10. Condition unimproved. Kernig's sign and rigidity of neck more marked. Patient delirious, but can be made to give rational answers to questions.

September 11. Leucocytes 12,200. 50 c.c. of turbid, white fluid containing meningitis cocci withdrawn by lumbar puncture and 15 c.c. *antimeningitis serum* injected into the spinal canal.

September 12. The note reads: "Twelve hours after the puncture and injection of the serum the temperature came down by crisis from 102.5° to 97° F.; the patient was quiet and sleeping soundly. The pulse was good and the respirations slow and regular. This morning the rigidity of the neck is lessened, the headache is less, the temperature subnormal, the respirations are regular and slow, the pulse slower (84) and of good volume, and the patient is improved in every way. The pupils are moderately dilated and the movements of the eyes good. There is a marked herpetic eruption about the mouth and three ulcers; one between fingers on the left hand and one on the left ear, and another beginning beneath the left eye. Leucocytes 8,750. "While counting the leucocytes six diplococci were found in a broken polymorphonuclear cell" (note by Dr. Longcope).

September 13. The temperature rose again yesterday, but came down during the night. This morning obtained 30 c.c. fluid by lumbar puncture and injected 15 c.c. *antimeningitis serum*.

September 14. Much better to-day. Rigidity of neck and Kernig's sign almost gone; temperature subnormal; is rational.

September 15. Improving, but paralysis of the left external rectus is noted.

September 16. The paralysis of external rectus more noticeable and slight rigidity of neck and Kernig's sign still persist. 35 c.c. of clear, colorless fluid containing a few whitish flakes but showing no diplococci were obtained on lumbar puncture. 15 c.c. of *antimeningitis serum* injected. Leucocytes 8,800. In the evening the temperature rose to 101°, the rate of respiration increased somewhat, but the pulse continued good (108). The rigidity of the neck and Kernig's sign were both increased, but they were much less marked than on admission.

September 18. Condition much improved. Neck rigidity and Kernig's sign have disappeared. Patient is hungry.

September 20. Temperature normal for three days.

September 24. Improving daily; house diet.

September 26. Out of bed.

September 30, discharged cured.

Discussion.—The onset of the disease in this case was abrupt, and the patient was admitted to the hospital about 48 hours after the appearance of symptoms. The diagnosis was established by the clinical symptoms and by lumbar puncture and a bacteriological examination on the third day of the disease, and a second puncture and an injection of the serum were made on the fourth day of the disease. It is worth noting that the circulating leucocytes fell to

one half the number between the first two punctures. A critical fall in the temperature took place after the second puncture and the first injection of the serum, and the general condition of the patient changed for the better while the rigidity of the neck muscles diminished. As the temperature rose slightly 72 hours after the first injection of serum a second puncture and serum injection were made. No further rise in temperature is recorded following the second injection of the serum. But as Kernig's sign and stiffness of the neck still persisted on September 16 and paralysis of the left external rectus appeared on the 15th, a third puncture and injection of serum were carried out. Since the leucocyte count was 8,800 on the day of the last injection complete previous cessation of active inflammation may be assumed, and the assumption is rendered certain by the limpid character of the spinal fluid withdrawn, from which all diplococci had disappeared. Forty-eight hours after the third puncture and serum injection all symptoms had subsided. The patient made a rapid and complete recovery.

Pennsylvania Hospital, Philadelphia. Service of Dr. Alfred Stengel.

CASE IV. C. B. White male, aged 11 years. Italian.

Present Illness.—The patient was admitted to the hospital on October 1 and had been sick since September 21. The onset was accompanied by vomiting, fever and chills. The complaint is of headache and pain in the back of the neck; the patient is delirious.

Physical Examination.—Half-grown, fairly well-nourished boy. He is very restless, requiring to be strapped in bed, and he cries out in delirium. Eyeballs are prominent; herpetic eruption about nose and mouth; tongue rough and covered with a brownish coat. The head is retracted and efforts to move it forward cause him to cry out. Suggestive Kernig sign; knee jerks unsatisfactory. Temperature on admission 101.2° F.

October 1. Leucocytes 10,500. 3 p. m. Lumbar puncture unsuccessful.—12.30 a. m. Second futile attempt to secure fluid by lumbar puncture. Patient very restless. Chloral and sodium bromide administered but ineffectual. Fairly quiet for 5 or 6 hours after ethyl chloride.

October 2, 4 p. m. Lumbar puncture yielded 6 c.c. thick, viscid, yellow pus. The last amount withdrawn thinner than the first. 10 c.c. of *antimeningitis serum* injected. Pus contains many meningitis cocci. Kernig's sign positive. Patient very restless; muscular twitchings.

October 3. Leucocytes twenty hours after puncture 14,750. No relief from puncture and serum. Three hours after puncture temperature rose from 101° to 104° F. It remained at 104°, except for a temporary drop to 102° through the night and the next morning. Marked nystagmus this morning. Pulse weak and rapid (130-160), respiratory rate has been steadily increasing; muscular

twitchings are very marked and the arms are jerked spasmodically. Stimulation increased, but pulse and respiration grow steadily more rapid and feeble.—4 p. m. Lumbar puncture yielded 12 c.c. turbid, purulent fluid. 15 c.c. of *antimeningitis serum* injected. Pulse and respiration gradually increasing. Temperature at 8.15, 106°. Unconscious and less noisy in delirium. Died at 1.45 a. m. (October 4). The autopsy (Dr. W. T. Longcope) showed acute cerebro-spinal leptomeningitis, acute broncho-pneumonia; chronic interstitial hepatitis; chronic interstitial splenitis; chronic fibrous pleurisy; congestion of intestine and hyperplasia of lymphoid follicles; and cloudy swelling of the kidneys.

Discussion.—The patient was admitted to the hospital on the tenth day of the disease. The puncture of the spinal canal indicated, and the autopsy proved, the cerebro-spinal meninges to be covered with a thick layer of pus cells and fibrin. No favorable influence was exercised on the course of the disease by the puncture and the serum injection carried out first on the eleventh and next on the twelfth day of the disease when the patient was already in an extreme condition.

Pennsylvania Hospital, Philadelphia. Service of Dr. Henry.

CASE V. P. C. Male, aged 18 years. Italian. Shoemaker.

Present Illness.—Patient came to the hospital on August 7 complaining of malaise, severe headache, chilliness and fever, nausea and constipation. The headache and malaise began on August 4 and increased so that he was compelled to stay in bed. On admission to the hospital the temperature was 101°, pulse 72, respirations 24.

Physical Examination.—Patient is moderately well-developed. He lies quietly in bed but is stuporous. Skin dry and hot; no rashes. Somewhat flushed and apathetic facies. Pupils equal and react sluggishly. Conjunctivæ injected. Tongue heavily coated and moist; breath fetid. The patient resents disturbance and becomes irritable; complains of pain in the head. (Nothing abnormal discovered in heart, lungs and abdomen. A blood examination showed Widal test negative and leucocytes 18,700. Slight degree of Kernig's sign present. Urine: no albumen, sugar or casts.

August 9. Temperature has almost reached normal; headache severe; there is marked rigidity of neck with slight retraction of the head. Some degree of rigidity of the back. The reflexes are exaggerated. Lumbar puncture was made at 1 p. m. but no fluid was obtained.

August 10. Temperature has risen. The condition of patient apparently worse; the stupor has increased and at times there is delirium. Rigidity of neck and Kernig's sign have increased; slight degree of nystagmus and strabismus. Lumbar puncture at 1 p. m. yielded 3 c.c. of spinal fluid tinged with blood. The centrifugated specimen showed polymorphonuclear leucocytes and red corpuscles, but no bacteria. Cultures were negative.

August 12. Condition essentially unchanged. The temperature fluctuated somewhat. Complains less of headache. Leucocytes 19,750. Lumbar puncture

at 1 p. m. yielded 4 c.c. of fluid of which 3 c.c. collected in one tube was slightly blood-tinged and 1 c.c. in another presented a silvery sheen. Cover-glass preparations showed many polynuclear cells, a few mononuclear cells and many red corpuscles, but neither tubercle bacilli nor diplococci. Cultures were sterile.

August 15. Patient slightly improved; brighter; temperature lower; still has considerable headache; persistence of neck rigidity and Kernig's sign; patellar reflexes exaggerated. Pulse good.

August 16. Condition about the same except that the temperature has become normal: Lumbar puncture at 1 p. m. yielded 7 c.c. of turbid spinal fluid. No tubercle bacilli found, but many pus cells and extracellular and smaller number of intracellular diplococci resembling the intracellularis, were seen. At 2.45 p. m. 15 c.c. of antimeningitis serum injected into the spinal canal. "Patient stood the ordeal well, one hour later felt more comfortable and was dozing."

August 17. Temperature remains normal. Patient feels much better; he is brighter; headache less severe; neck less rigid, relaxing; Kernig's sign diminished.

August 19. Patient Convalescing. Headache slight; rigidity less; appetite improved.

August 22. Condition good; no headache; no rigidity; reflexes normal. Sitting up.

August 30. Discharged cured.

Discussion.—The diagnosis in this case must be accepted chiefly, if not entirely, upon the basis of the symptoms. The bacteriological examination of the fluid yielded by lumbar punctures does not supply convincing proof of the existence of epidemic meningitis. The degree of general leucocytosis is in agreement with what is commonly found in epidemic meningitis, but the evidence supplied by the leucocyte count is circumstantial. The examination of the sediment obtained from the spinal fluid showed an excess of polymorphonuclear cells, but it was not until the 16th instant, or on the tenth to twelfth day of the disease, that turbid spinal fluid, containing many leucocytes and stainable diplococci, was secured. It does not appear in the report that the cocci were certainly identified as *Diplococcus intracellularis* by culture tests. Hence the question remains open whether in this case the general cerebro-spinal fluid remained free of the diplococcus although an exudative inflammation of some extent existed in the membranes. The case appears to have been progressing towards recovery before the injection of the serum on the tenth to twelfth day of illness, and there is lacking all certain evidence that the progress in this direction was accelerated appreciably by the serum. Total amount of serum injected, 15 cubic centimeters.

RESULT OF THE USE OF ANTISERUM BY DR. CUSHING, OF BALTIMORE.

Dr. Cushing wrote one of us (Flexner) as follows:

"I remember your telling me that you thought there was very little chance of benefit to be expected from the serum in other than the acute stages of epidemic meningitis. It may, therefore, interest you to know of this single experience. A woman, aged 36 years, had a severe attack of cerebro-spinal fever with sudden onset, May 16, 1907. She was very ill and during the next few weeks Fletcher and a number of others saw her in consultation. The condition dragged on until July, when I was asked to see her in the hope that there might be some prospect of operative relief, since she was still suffering from irregular periods of fever (103° – 104°) during which there were marked stupor, severe headache, and cervical retraction. She had been ill in all about eight weeks. Though under the impression that there was some mechanical obstruction with hydrocs ventriculorum, a lumbar puncture during one of her stuporous periods evacuated a large amount of not particularly turbid fluid which was under high tension. To my astonishment the fluid was swarming with *Diplococcus intracellularis*, both inside and outside of cells. Forty-eight hours later, on the return of the stupor, another puncture was made and 15 c.c. of *antimeningitis serum* were introduced into the spinal canal; the diplococci were still numerous. The temperature dropped to normal soon after. After a second forty-eight hours *this was repeated*; diplococci were few. Again in forty-eight hours there was a repetition of the puncture and *serum injection*; practically no diplococci could be found in the fluid, though there may have been a few organisms present. From this time on there were no further symptoms—no headache and no fever. She rapidly convalesced and has recovered her usual health."

Discussion.—The instructive points of this case relate to the persistence of the diplococcus in large numbers in the cerebro-spinal membranes for a number of weeks after the acute stage of the meningitis had passed off, and the action of the lumbar punctures and serum injections in interrupting and finally and quickly abating the free development of the diplococcus. Apparently the number of diplococci present at the second puncture, before any serum was injected, was not remarkably smaller than at the first puncture, while the next two notes describing the punctures and injections of serum dwell particularly on the diminution in numbers of the diplococcus. The subsidence of symptoms and return to perfect health were undoubtedly attendant upon and probably the outcome of the disappearance of the diplococcus from the cerebro-spinal membranes, and this disappearance was greatly aided by, and possibly accomplished through, the withdrawal of infected spinal fluid and the injection of the antiserum.

THE EPIDEMIC OF MENINGITIS IN GREAT BRITAIN.

A severe epidemic of cerebro-spinal meningitis, caused by *Diplococcus intracellularis*, prevailed in several cities in England, Scotland and Ireland during the winter of 1906-7, and is still prevailing in those countries. We have been fortunate in securing the co-operation in testing the antiserum of Dr. Claude Ker, of the City Hospital of Edinburgh, and of Dr. A. Gardner Robb, of the Belfast City Fever Hospital and the Belfast Union Fever Hospital, of Belfast. We have received preliminary reports, based on a small number of cases of meningitis treated with the antiserum, from these gentlemen which are given in this place. They will doubtless publish their experiences in full later, after a larger number of cases, treated with the antiserum, have come under their observation.

Dr. Ker writes under the date of September 17, 1907:

"Our experience with the serum has so far been limited to four cases. The first three have done well and seem now, after four and three weeks' treatment in hospital, to be out of danger. Our general impression here is that, although averagely acute cases, they have done most exceptionally well. One, however, I fear will be permanently deaf.

"The fourth case was fulminant with profuse hæmorrhages in the skin. It was admitted 18 hours from the onset and died about 24 hours from the onset. It was therefore hopeless, but I thought it just and right to give it the chance, and it had one dose of 30 c.c. of the serum, which could hardly have had time to be effective. The other cases had about 120 c.c. on an average each. The only selection I am making is to treat cases which are less than a week old.

"By the way, my bacteriologist tells me that it is exceedingly difficult to get the diplococci to stain as usual the day after the first intraspinal injection. This has not been noticed so much with other serums. The germs are there of course and can be seen, but they must be modified in some way by the treatment, as they certainly lose their staining power."

Dr. Robb writes under the date of October 23, 1907:

"Since my return to duty on 1st September the opportunities of testing the serum have not been many, but the results in the cases receiving it have been remarkably satisfactory. I have only had four cases admitted to hospital quite early in the attack since that time.⁴

"The first case was a man of 22 who was 48 hours ill when admitted. He was wildly delirious with normal temperature, cyanosis and plentiful petechiæ. I considered his case practically hopeless from my former experience. I gave him

⁴ In addition, two chronic cases of meningitis were treated, as appears from the latter part of the letter.

30 c.c. of the serum after drawing off 90 c.c. of turbid fluid in which the meningococcus was present. 36 hours later he was quite conscious and had no headache; his temperature rose to 101° F. and remained about that level for some days; he had very abundant herpes, but his symptoms rapidly improved. I repeated the 30 c.c. injections at intervals of 3 days, giving 90 c.c. in all. He made a complete recovery. The remarkably sudden clearance of the mental symptoms and the complete disappearance of the headache were very striking, and as this has taken place in other cases treated with the serum, I think the serum must have the credit.

"The second acute case was a girl of 12 years with very severe attack admitted on the second day. This case was very severe, but the prognosis without serum would have been uncertain. The same good results followed with good recovery.

"The third case, a woman of 31, had been ill 10 days with very severe attack; high temperature, delirium, and very marked rigidity. My prognosis would have been bad, but she rapidly improved after the serum and is in a fair way to recovery now. She had 90 c.c.

"The fourth acute case, a man of 20, with maniacal delirium, came in two days ago. I gave him 30 c.c. of serum immediately on admission—then 46 hours ill. The cerebro-spinal fluid was quite purulent even then. He died of heart failure some 9 hours after receiving the serum.

"Even more striking were the results in two chronic cases. One boy not doing well, who had continuous fever for 35 days, received 30 c.c. of the serum. From that time on he made steady improvement, had no further headache and has made a complete recovery. Another case which I considered quite hopeless got 30 c.c. on the 25th, and again on the 32d day. He made steady improvement from the first dose and is now up. One young child, who was also hopeless, showed no improvement and died, but in this case thick, stringy pus was obtained. In the chronic cases no improvement had followed simple drawing off of fluid.

"I quite appreciate how dangerous it is to draw conclusions from a few cases, especially during a lull in the epidemic, but allowing for all that I am greatly impressed with the results in the cases I have had. I believe there has been little if any change in the virulence of the type here. The cessation of the headache in the chronic cases receiving serum and in whom it had been most troublesome was very striking; and the absence of headache in the acute cases after serum I have not seen in any other cases."

Discussion.—There could be no advantage gained in discussing these cases, since they are reported so very briefly. Attention may, however, be called properly to the effects of the serum injections in the two cases of the chronic disease described by Dr. Robb. They recall the similar effects of the serum injections made in the chronic case of epidemic meningitis described by Dr. Cushing, of Baltimore.

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ST. VINCENT'S HOSPITAL CASES, NEW YORK CITY.

The antiserum was not available for use in the treatment of meningitis during the prevalence of the severe epidemic in New York City. Since it has been available only occasional sporadic cases of the disease have continued to appear, and many of these have entered hospitals late in the course of the disease. Hence our opportunities for testing the antiserum under conditions of personal observation have been very few. We have secured from Dr. Strain, of St. Vincent's Hospital, the records of three cases of meningitis in adults caused by *Diplococcus intracellularis* of which abstracts follow.

CASE I, No history of onset obtained; total serum injection 30 c.c.; death.

CASE II, 1st injection of serum on 4th day; total serum injection 30 c.c.; recovered by lysis.

CASE III, No history of onset obtained; total serum injection, 60 c.c.; recovered by lysis.

St. Vincent's Hospital, New York. Service of Dr. Lewis.

CASE I. A. C., aged 22 years. Greek.

Present Illness.—No history could be obtained. Admitted to hospital, April 23, 1907.

Physical Examination.—The patient is in a semi-comatose condition. Pupils unequal; right pupil does not react to light; left is dilated; no strabismus. Herpes on upper lip. Marked retraction of the head and stiffness and tenderness of neck. Reflexes absent from extremities; Kernig's sign present. Babinski reflex absent.

April 23. Patient is somnolent, pulse slow and of medium tension; general condition poor. Temperature ranged from 102°–100°.

April 24. Patient noisy, crying out. No remarkable change. Leucocytes 18,000.

April 25. General condition about the same. 15 c.c. of turbid spinal fluid obtained by lumbar puncture. Microscopical examination showed pus cells and *Diplococcus intracellularis*.

April 26. Condition worse; the pulse is rising gradually and the first heart sound is becoming weaker; capillary circulation very poor; cyanosis; cold extremities. Temperature 100°–103°; pulse 99–112; respirations 28–34.

April 27. Condition about as yesterday. 20 c.c. of turbid spinal fluid removed by lumbar puncture and 30 c.c. of antimeningitis serum injected. Following this the temperature rose 1° and then gradually declined during the night to 99°, the patient's condition gradually becoming worse.

April 28. Condition very bad; pulse hardly perceptible; respiration accelerated; physical signs unchanged.

April 29. Lumbar puncture unsuccessful. Condition bad.

May 1. Death at 1 a. m.

Discussion.—The duration of the disease in this case is not known. The diagnosis of epidemic meningitis, which was suggested by the symptoms, was established by lumbar puncture on April 25, two days after admission to hospital. The second puncture and the first injection of the serum were made on April 27, four days after admission to the hospital and at a time when the patient's condition was already very poor and the circulation had begun to fail markedly. No improvement followed the puncture and injection, but the patient's condition gradually grew worse until his death on May 1, eight days after admission to the hospital, four days after the serum injection, and an unknown period after the onset of the disease. Total amount of serum injected, 30 cubic centimeters.

St. Vincent's Hospital, New York. Service of Dr. Lewis.

CASE II. C. L. Aged 22 years. Greek. Laborer.

Present Illness.—On May 7 (one day before admission to hospital) the patient was taken suddenly ill with sharp chill and intense headache. The neck became stiff, and slight movement greatly increased pain in head and neck. There were fever and photophobia.

Physical Examination.—Flushed face; anxious expression, injected conjunctivæ; photophobia; beginning herpes on nose and lips; tongue coated; breath foetid. Neck stiff and head retracted. Kernig's sign marked; reflexes absent; skin hyperæsthesia.

May 8. Temperature 102°, pulse 90, respirations 24 on admission. Patient quiet, but complains of headache. Under ice cap slept much of the day.

May 9. Patient somnolent; cries out at times. Temperature ranged from 103.4° to 105°; pulse 80-94.

May 10. At 9 a. m. and 1 p. m. temperature 104°.—4 p. m., lumbar puncture yielded 35 c.c. of turbid fluid containing pus cells and large numbers of diplococci. Headache better; slept much of night.—5 a. m. Temperature 100°.

May 11, 4 p. m. Lumbar puncture; 30 c.c. spinal fluid withdrawn and 30 c.c. of antiserum injected. During the night patient was very noisy and required sedatives (morphia and hyoscin) to give sleep.

May 12, 9 a. m. Temperature 104°; remained high all day. No marked change in the condition.

May 13, 5 a. m. Temperature 100°. Pulse slow and full; mental condition apathetic. Temperature did not rise above 101°. Involuntary bladder and bowel movements.

May 14. Condition improved; mental condition brighter. Patient follows with the eyes persons and objects about the room.

May 15. No marked change. Temperature ranged from 101.8° to 103°.

May 16. Condition better. Awake greater part of day; headache less; does not complain of pain in the neck.

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May 17. Improvement continues; patient comfortable; mentally brighter; less rigidity of neck; appetite improved.

May 19. Temperature normal; neck rigidity and headache almost gone; patient bright and cheerful.

The improvement in the patient's condition continued and he was discharged cured on June 8.

The leucocyte count on three occasions gave 11,000, 15,000 and 14,000.

Discussion.—The onset of the symptoms in this case was sudden and by the end of the first twenty-four hour period the disease had reached full development. On the third day, counted from the appearance of the first symptoms, lumbar puncture was made and the diagnosis established by bacteriological examination. The temperature fell, the headache diminished, and the patient rested better than before. On the fourth day a second puncture was made and the antiserum was injected. No immediate favorable effect followed, but 60 hours later the condition had improved and there was a steady subsequent improvement up to complete recovery. Convalescence may be said to have begun not later than the tenth day of the disease. Total amount of serum injected, 30 cubic centimeters.

St. Vincent's Hospital, New York. Service of Dr. Lewis.

CASE III. G. P. Aged 18 years. Greek. Clerk.

Present Illness.—No definite history obtainable. About a week before admission to hospital on May 18, 1907, he complained of headache and vomiting.

Physical Examination.—Face flushed and expresses pain; photophobia; pupils equal and react to light; tongue coated and dry; neck rigid and head retracted; a few petechial spots on thorax, abdomen and legs; reflexes exaggerated; no clonus or Babinski; Kernig's sign marked. May 18 the temperature rose from 98.5° to 103°; pulse 85; respirations 28.

May 19–28. The condition has not greatly altered. The mental condition dull, there is much headache and at times delirium requiring restraint. The temperature did not rise above 102.5° and ranged about 101°. Leucocytes 24,000.

May 27. Leucocytes 21,000.

May 29. Patient quiet and somnolent. By lumbar puncture about 50 c.c. of turbid fluid were obtained and 30 c.c. of antiserum injected. Following the injection the patient was quiet and the temperature fell to 99°. Microscopical examination of spinal fluid showed pus cells and intra- and extracellular diplococci, which were cultivated.

May 30 and 31. Mental condition somewhat improved; no delirium.

June 1. External strabismus appeared, otherwise no change.

June 2. Mental condition clearer; patient answers questions. Temperature fluctuating.

June 4. Patient complains of pain in neck and is at times somewhat delirious. About 50 c.c. of spinal fluid removed by lumbar puncture and 30 c.c. *antimenigitis serum* injected. The temperature fell from 103° to normal in twenty-four hours. Delirium subsided and patient looked brighter. He had involuntary defecation and micturition.

June 6. Leucocytes 20,000.

June 7. Patient noisy at night, requiring sedatives.

June 10. Pulse weak but responds to digitalin.

June 11. Neck rigidity lessened; patient fairly comfortable; photophobia and strabismus gone; diet increased; temperature normal.

June 15. Patient gradually getting stronger; mental condition good.

June 18. Improvement continues.

June 21. Patient noisy and delirious all day; temperature 99°. For the next two or three days he was delirious at times.

June 27. Patient rational all day and slept and ate well. Temperature 97°.

June 29. Patient sat up for the first time.

July 5. Walked a few steps; leucocytes 6,000.

July 20. Discharged cured.

Discussion.—It is impossible to determine accurately the duration of the disease before the patient entered the hospital. On the eleventh day after admission lumbar puncture was made and a serum injection was given. Following these the patient's mental condition improved somewhat, but three days later external strabismus appeared. On the seventeenth day of illness a second lumbar puncture and serum injection were made which were followed within twenty-four hours by a marked fall in temperature and an improvement in the patient's mental condition. The temperature remained about normal afterwards, although there reappeared off and on for a few days, during the convalescence, a state of temporary delirium, which did not interrupt the general course of improvements in the patient's condition. Recovery was complete. Whether the serum injections influenced favorably the progress of the disease in this case cannot be determined with certainty. Total amount of serum injection, 60 cubic centimeters.

MANNER OF ACTION AND OF EMPLOYING THE ANTISERUM.

The plan to administer the antiserum by direct inoculation into the spinal canal in human beings was based upon the observations made by one of us (Flexner) on the bactericidal effect of normal sera and sterile exudates upon *Diplococcus intracellularis in vitro*,

and upon the curative action of antidiplococcus sera in guinea-pigs and monkeys infected with the diplococcus, when brought into immediate relation with the focus of infection. In view of certain theoretical objections to the employment as curative agents of antisera developed for a microorganism whose toxic action is caused by endotoxin, Flexner dwelt on the encouraging circumstance that in epidemic meningitis the main pathological lesions can be brought directly under the influence of the antiserum by injecting the latter into the spinal canal; and he pointed out that while it is undoubtedly important to secure neutralization of the endotoxin yielded by the diplococcus on disintegration, the effect of restraint of growth and multiplication of the diplococcus may, at some period of the disease, be of even greater significance.⁵ There is experimental evidence for the view that the antiserum possesses a certain antitoxic value since it can neutralize the toxic substances contained in autolysates of the diplococcus. But its power to bring about rapid suppression of the diplococcus in infected guinea-pigs and monkeys is considerable. In monkeys which have been injected with mixtures of emulsions of the diplococcus and immune serum simultaneously, or first with the emulsion and next with the immune serum, the diplococcus is caused rapidly to diminish in numbers and to be more abundantly taken up by leucocytes.⁶ Since the facts at hand do not warrant us in concluding that any considerable multiplication of the diplococcus takes place in the experimental infections, the power of protection of the antiserum would appear to be dependent upon the restraint which it exercises over all multiplication and the increased tempo of phagocytic inclusion of the diplococcus which it brings about. It is probable that phagocytic digestion not only prevents further multiplication of the diplococcus but also that it detoxicates the endotoxin by reducing it to simpler and non-toxic or less toxic compounds. Still, in a few instances, in which the antiserum was injected into the spinal canal of monkeys infected with the diplococcus, the microorganisms disappeared without marked phagocytosis and more slowly than in the cases in which outpouring of

⁵ *Jour. of Exper. Med.*, 1907, ix, 138.

⁶ *Idem*, p. 169 *et seq.*

leucocytes was considerable. The control of the pathological conditions in these instances appeared to depend less upon the phagocytes than upon the spinal fluid reinforced by the antiserum; and as the symptoms of intoxication were less than would have been present had the antiserum not been injected, a degree of antitoxic power must be ascribed to the antiserum.

If we turn to our knowledge of the manner in which the antiserum acts in controlling or modifying the infection in human beings we find ourselves possessed of very few facts. The observation recorded by Dr. Cushing indicates that the antiserum has the property of bringing about rapid diminution in the number of diplococci present in the cerebro-spinal fluid. Dr. Ker observed rapid rise of the opsonic index for the diplococcus following upon an antiserum injection and a modification and reduction of the staining power of the diplococcus in film preparations prepared from the meningeal exudate obtained by lumbar puncture. Others have noted this reduction in number and change in the staining properties of the diplococcus after the serum injections.

We have had the opportunity to follow in two young children the immediate effects of the antiserum injections on the number, appearances and viability of the diplococcus in the cerebro-spinal fluid. One of the children was 18 months old and had been two weeks ill when first injected at the Babies Hospital in New York. The spinal fluid withdrawn before the serum injection was slightly turbid and showed a fair number of extracellular and a large number of intracellular, sharply-staining diplococci. Cultures were easily secured from this fluid on blood and sheep-serum agar media. A second puncture made twenty-four hours after an antiserum injection yielded a fluid of the same appearance as before, but no extracellular diplococci, or very few, were contained in it, and the number of intracellular diplococci was much reduced. All the diplococci were more or less changed; they were swollen or fragmented and stained diffusely. Cultures were now negative. A second antiserum injection was given and the next day the diplococci had undergone a still greater reduction in numbers and continued to stain feebly. No cultures could be obtained from this fluid or any subsequent fluid from this child, although several later punctures were made.

The second child was two years old and had been ill about five days when admitted to the Presbyterian Hospital in New York under Dr. Northrup. The first lumbar puncture yielded a sero-purulent fluid containing large numbers of diplococci outside and inside pus cells. Abundant cultures were easily obtained. An injection of 15 cubic centimeters of antiserum was given and twenty-four hours later the lumbar puncture yielded a fluid in which a little blood obscured the color, but the pus cells and diplococci were diminished in numbers. The latter were now almost wholly inside cells and of irregular size and contour and weak staining power. A portion of the fluid was centrifugalized and the sediment used for preparing cultures which did not grow. Serum injection and lumbar puncture were repeated on two later occasions. The spinal fluid withdrawn was far less purulent than it had been, the diplococci became very few in number, and they did not again multiply on culture media otherwise favorably adapted for the growth of the diplococcus.

These observations, few in number as they are, go to show that the antiserum exerts a definite and injurious influence upon the diplococcus in the cerebro-spinal fluid through which its multiplication is restrained and it is rendered more subject, possibly, to phagocytic inclusion and digestion, at the same time that it is deprived of its capacity to grow outside the body on culture media.

We have given the following general instructions for the use of the serum:

The antiserum should be kept in a refrigerator until it is to be used, when it should be warmed to the body temperature before it is injected.

The antiserum is to be introduced directly into the spinal canal after the withdrawal of cerebro-spinal fluid by means of lumbar puncture.

The quantity of antiserum to be used at a single injection should not exceed, for the present, 30 cubic centimeters. It is desirable, although it would not appear to be essential, to withdraw from the spinal canal at least as much fluid as the amount of antiserum to be injected. The injection should be made slowly and carefully to avoid the production of symptoms due to increased pressure. This

precaution should be exercised especially where the quantity of cerebro-spinal fluid withdrawn is less than the amount of antiserum to be injected.

The injection of the antiserum should be repeated every twenty-four hours for three or four days or longer. Whether any advantage will be gained by more frequent or more numerous injections than here indicated a wider experience must decide. As much as 120 cubic centimeters of the antiserum have been injected into the spinal canal in four days without causing unpleasant symptoms.

The evidence at hand indicates that the earlier in the course of the disease the injections are made the better the results. Hence should the film preparation prepared from the first fluid obtained by spinal puncture show Gram-negative diplococci, some of which are within leucocytes, an injection of the antiserum should be made immediately and without waiting for the result of culture tests. Should the diagnosis be left in doubt or the disease prove later to be of another nature than epidemic meningitis, no harm will have been done by the injection of the antiserum.

Although the best results have thus far been obtained where the antiserum has been injected early in the disease, yet the serum should be used in its later stages also until our knowledge governing the value of the serum becomes more precise. The indications at present are that it is useless to employ the serum in the very late stages of the disease in which chronic hydrocephalus is already developed.

Precise records of the manner of action of the antiserum upon the general symptoms of the disease and the local inflammation and the diplococcus should be kept. Information is greatly desired on the influence of the antiserum upon the number, appearances, growing properties, etc., of the diplococcus, upon the relation of the diplococcus to phagocytosis, and on the number and appearances of the leucocytes, before and after the antiserum injections. Counting the leucocytes in the circulating blood, before and after the injections, would help determine whether the antiserum tends to bring a greater number of leucocytes into the inflamed membranes, or whether it leaves the number unchanged or causes cessation of the emigration.

Until the antiserum is proven to be of value or of no value in the treatment of epidemic meningitis its manner of action should be carefully observed and recorded so that a definite decision may be reached as quickly as possible.

DOES ANAPHALAXIS OCCUR FROM REPEATED INJECTIONS OF THE SERUM?

That the human organism reacts more vigorously to second and subsequent injections of horse serum than to the first injection is shown by the reports of many instances in which these stronger effects were noted after administering diphtheria antitoxin. v. Pirquet and Shick⁷ call this condition of greater reaction on the part of the animal organism "serum-disease." Wolf-Eisner⁸ sees in this state of intensified effect or hypersensibility the fundamental pathological condition underlying the manner of reaction of the animal body to repeated injections of foreign proteids in general, including the bacterial endotoxines. Our precise knowledge of serum-hypersensibility—or anaphalaxis—is due to the impulse given the study of the subject by Theobald Smith and to the exact studies of Otto,⁹ Rosenau and Anderson,¹⁰ Besredka and Steinhardt,¹¹ Gay and Southard,¹² Lewis¹³ and others. The particular fact that concerns us at this moment is whether a possible danger to the patient is to be feared from intraspinal injection at considerable intervals of a foreign serum. We know that the intensified effects in man of repeated serum injections under the skin causes discomfort but does not menace life. Besredka and Steinhardt have, however, shown that it is precisely the direct inoculation of the central nervous system with the alien serum in a hypersensitive guinea-pig that is to be feared. It is, therefore, of the first importance to us to ascertain whether a similar danger exists in relation to the intradural injection of the antimeningitis serum.

⁷ Die Serumkrankheit, Vienna, 1905.

⁸ Berl. Klin. Woch., 1907, xliv, 38.

⁹ Leuthold-Gedenkschrift, 1906, i, pt. 1, 153.

¹⁰ U. S. Marine Hosp. Service Hygiene Lab. Bull., 1906, No. 29.

¹¹ Annales de l'Institut Pasteur, 1907, xxi, 117, 384.

¹² Jour. of Medical Research, 1907, xi, 143.

¹³ Jour. of Exper. Med., this number.

There is no danger, apparently, to be apprehended from a single injection of even a considerable volume of the serum into the spinal canal. Daily intradural injection of the antiserum seem also to be well borne, at least, for several days. The question arises whether it is safe to give the injections at intervals of many days, since an interval is necessary in order that the reaction of hypersensibility shall be developed. Dr. Ladd's Case V, a child of three years, was injected on the following dates: April 1, 6, 22; no ill effects followed and recovery was complete. His Case XV, a child of eight years, was injected as follows: June 16 intradurally, June 18 idem, June 21 subcutaneously, June 25 idem, June 27 idem, July 10 intradurally; no ill effects followed and the child recovered. Still other instances of repeated injection, with intervening long interval between certain injections, will be found among the recorded cases. The danger does not, therefore, seem to be great.

We wish now to refer to an infant who was injected at the Babies Hospital, New York City, several times with the antiserum. The fourth injection was made 42 days after the first and 16 days after the third injection and was followed by convulsions, prolonged rigidity and elevation of temperature.

Babies Hospital, New York. Service of Dr. L. Emmett Holt.

E. F. Female child, 11 months old. About the middle of August developed fever, hyperæsthesia, rigidity and projectile vomiting. Admitted to Babies Hospital September 6, 1907. Lumbar punctures on September 7 and 11: turbid fluid withdrawn. September 12, 45 c.c. fluid withdrawn and 5 c.c. *antiserum injected*. September 17, 25 c.c. fluid withdrawn, no injection. September 28, 7 c.c. fluid withdrawn and 7 c.c. *antiserum injected*. October 8, 3 c.c. fluid withdrawn and 5 c.c. *antiserum injected*. No symptoms developed following these injections. October 24, 60 c.c. fluid were withdrawn and 20 c.c. *antiserum injected*. Previous to the last injection the child was relaxed and opisthotonos was absent. She was restless and irritable, vomited occasionally, cried if disturbed, and was apparently deaf. The reflexes were increased and there was marked emaciation. The antiserum was administered intradurally at 11 a. m. At 3 p. m. a severe convulsion occurred and marked hyperextension and opisthotonos developed. These were still present on October 29. The temperature before the injection was about 98 to 99°; for four days after the injection it rose to 102° and once reached 104°.

The conditions which developed in this child following the last injection of serum, after an interval of 16 days since the preceding injection, cannot be explained readily. We prefer to leave the

question open whether the phenomena belong to the anaphalactic state or are of another nature. But so far as the case bears on the general question of the intradural injection of the serum it has theoretical rather than practical significance. The antiserum will, as a rule, discharge its beneficial effects in the first days of its employment and for this no danger is known to exist. Rarely, after a resting period in its use, a relapse of the acute infection may call for another injection. The records of the use of the antiserum in relapses do not show that any ill effects followed the injections. We think that the reinjection of the serum in supposed relapses should be based upon demonstrated reappearance of or increase in the diplococcus, since mere sudden rise in the temperature, in the course of meningitis, may obviously be due to other causes than a reinvasion of *Diplococcus intracellularis*.

MANNER OF PREPARING THE ANTISERUM.

The antimeningitis serum employed in the treatment of the cases of epidemic meningitis described in this paper has been made in the horse. The general method of preparation has been as follows:

The first inoculation consisted of cultures of the diplococcus, heated to 60° C. for 30 minutes, injected under the skin. Many different strains of the diplococcus were combined to prepare this vaccine. The first dose was the equivalent of $\frac{1}{4}$ surface growth on sheep-serum agar in a test tube. The dose was doubled at each subsequent inoculation, until an amount equal to four test tube growths could be given at 5 to 7 day intervals.

Intravenous inoculation was now substituted for the subcutaneous. Beginning with one oese of living diplococci the dose was progressively increased to 2, 3, 5, etc., oese, then $\frac{1}{2}$, $\frac{3}{4}$, 1, etc., agar slant cultures, and finally to 1½ bottles (12 oz. Blake) of surface growth. The larger quantities of the culture injected into the vein caused such severe reactions and alarming symptoms that they were discontinued.

Subcutaneous and intravenous injection of an autolysate¹⁴ was now used. The doses, at first 1 cubic centimeter, were later increased to 3 cubic centimeters. The injections were made about one week

¹⁴ *Jour. of Exper. Med.*, 1907, ix, 105.

apart. The intravenous injection of the autolysate was discontinued because of the serious symptoms (increased respiration, weakness, etc.) which resulted from them.

At the present time the subcutaneous tissues are being used exclusively for the inoculations, which are made alternately of living diplococci and autolysate at 7-day intervals. Many different strains of the diplococcus are employed in preparing the living cultures and the autolysate for inoculation. The dose of living diplococcus has been increased to one and one half bottles, and of autolysate to the equivalent of one and one half bottles of the cultures.

The febrile reaction to the subcutaneous inoculations is moderate: The temperature rises to 39° to 39.6° . The animal eats less during the febrile period. The local reaction is much greater. Within a few hours a swelling appears at the site of inoculation and extends widely—from the shoulder to the knees at times. The swelling tends to disappear in a few days or, in those instances in which the larger doses of living diplococcus or autolysate are used, sterile abscesses develop which eventually discharge through the skin.

The antiserum has been titrated by the complement-deviation method devised by Kolle and Wassermann,¹⁵ and tested against the autolysate in guinea-pigs. Neither method appears to be quantitatively accurate. The antiserum used in the treatment of the cases of epidemic meningitis described in this paper came from a horse in process of immunization one year or longer before being used for supplying the serum.

Coincidentally with our efforts to produce an antidiplococcus serum for use in human beings suffering from epidemic meningitis, Kolle and Wassermann¹⁶ and Jochmann¹⁷ attempted in Germany to prepare such a serum. Only brief reports of the employment of these sera in the treatment of epidemic meningitis have thus far appeared in print. Wassermann¹⁸ has recently reported the results of the treatment of a series of cases of the disease by subcutaneous injections chiefly of his serum, with results which, on the whole, appear

¹⁵ *Deut. med. Woch.*, 1906, xxxii, 16.

¹⁶ *Ibid.*

¹⁷ *Ibid.*, p. 20.

¹⁸ *Ibid.*, 1907, xxxiii, 1585.

to be favorable to the value of the treatment. Schöne¹⁹ treated a still smaller series of cases with Jochmann's serum, partly by the subcutaneous and partly by the intradural method of injection, with results said to have been beneficial, especially where the injections were made into the spinal canal.

GENERAL DISCUSSION.

In view of the small number of cases of epidemic meningitis upon which this paper is based, it would seem the wisest policy, possibly, to defer all general discussion of the results until such time as a larger series of cases treated with the antiserum having been collected, a searching analysis can be made.

On the other hand it seems desirable to recapitulate, at this time, the salient points brought out by the different reports which have been collected in this paper, it being understood that they do not represent conclusions but merely statements of observed facts or obvious deductions from them.

Thus, for example, the records of cases used in the preparation of this paper show that of 47 cases of epidemic meningitis treated with the antiserum, 34 recovered and 13 died. Expressed in percentages, the recoveries equal 72.3 and the deaths 27.6 per cent. The records also show that of the 13 fatal cases four were either fulminant in type, in which death took place within 24 to 36 hours of the onset, or the patient's condition was so extreme that death occurred in a few hours of the injections of the antiserum. If, therefore, these four cases are subtracted there results a total of 43 cases of meningitis treated with the antiserum of which 34 recovered and 9 died, or 79.9 per cent. recoveries and 20.1 per cent. deaths.

This tabulation takes the cases without respect to their duration at the time the treatment was begun. This is manifestly a severe test, but so long as the whole number of cases is so small no other mode of analysis is likely to be as useful. Should it be desired, however, to know the ratio of recoveries to deaths in cases injected with the antiserum in the first three days of illness, the calculation could be based on 18 cases of infection, exclusive of the ful-

¹⁹ *Die Therapie der Gegenwart*, 1907, xlviii, 52.

minant ones, of which 16 or 88.9 per cent. recovered and 2 or 11.1 per cent. died. .

In order that these figures should have any value whatever we are obliged to know with what degree of severity the epidemic prevailed at the time and place at which the antiserum was used. We possess, fortunately, good data concerning this point. Eighteen cases of epidemic meningitis occurred at Castalia within a few weeks. Of these twelve patients died and six recovered. Of those patients who recovered three were injected with the antiserum, and no patient having received the serum died.

The report from Akron covers twenty cases of epidemic meningitis of which nine were not treated with the antiserum: Eight died and one recovered. The remaining eleven cases were treated with the antiserum: three died and eight recovered. The three fatal cases included two of the fulminating variety.

Dr. Robb²⁰ has given statistics covering 230 cases of epidemic meningitis which arose in Belfast. Of these 162 died making a mortality of 70.43 per cent. As regards the severity of the disease at the time at which the antiserum prepared by us was employed he states in his letter (p. 203): "I believe there has been little if any change in the type here."

The majority of the cases which were treated with the antiserum were in children over five years of age and in adults. Since the mortality is highest among young children a table has been prepared, from Dr. Ladd's report, giving the results of the use of the serum in children under five years of age (p. 185). Of the eight children belonging to this group seven were under three years and one was about five years old. Five of the children recovered and three died. Two of the three fatal cases were injected on the fourth day and one on the twentieth day.

The records of the patients who recovered have been full enough in twenty-five instances to enable us to make out the manner of termination of the disease—whether by lysis or by crisis. We have found that thirteen times the disease terminated by lysis and twelve times by crisis. The accompanying table (Table I) illustrates this point and enables the individual case-histories, upon

²⁰ *British Medical Journal*, 1907, No. 2443, 1129.

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TABLE I.
Dr. Ladd's Cases.

Case No.	Day of Disease. First Injection.	Total c.c. Anti- serum Injected.	Recovered by Lysis.	Recovered by Crisis.	Died.
I.	3	25	+		
II.	12	5		+	
III.	4	30	+		
IV.	5	15			+
V.	2	35		+	
VI.	1	15		+	
VII.	3	20		+	
VIII.	3	45	+		
IX.	2	35			+
X.	1	15		+	
XI.	20	25			+
XII.	4	46			+
XIII.	1	45		+	
XIV.	14	28	+		
XV.	3	53	+		
XVI.	4	41			+

Akron Cases.

I.	5	82.5	+		
II.	1	10			+
III.	12	20	+		F
IV.	2	43.5	+		
V.	7	25		+	
VI.	2	22.5			+
VII.	1	15		+	
VIII.	1	35			+
IX.	2	105	+		F
X.	1	25		+	
XI.	2	22.5		+	

Pennsylvania Hospital Cases.

I.	4	15		+	
II.	3	25	+		
III.	4	45		+	+
IV.	11	25			
V.	10	15	+		

St. Vincent's Hospital Cases.

I.	?	30			+
II.	4	30	+		
III.	?	60	+		

Edinburgh Cases. Recovered (no details).

I.	—7	120	+		
II.	—7	120	+		
III.	—7	120	+		
IV.	1	30			+

Belfast Cases.

I.	2	90	+		
II.	2	90	+		
III.	10	90	+		
IV.	2	30			+ F
V.	35	30	+		
VI.	25	60	+		
VII.	?	?			+

Dr. Cushings' Case.

I.	56	45	+		
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— = less than.

F = Fulminating.

which it is based, to be scrutinized. In a number of cases the abrupt termination of the disease, after acute and violent onset, within forty-eight hours of the injection of the antiserum, was striking and impressive. In some cases the serum was injected as early as twelve hours, and in one case as early as two hours, after the onset of severe symptoms, with prompt arrest of the disease.

Once or twice after abrupt arrest of active symptoms lasting several days, relapses occurred which were as promptly controlled by another injection of serum as was the original infection. It would appear that during the height of an epidemic of meningitis spontaneously abortive cases are of infrequent occurrence.

To discuss, on the basis of so small a series of cases as is here presented, whether the antiserum can be said certainly to influence favorably the temperature, mental condition, and such special symptoms as headache, muscular rigidity, paralyses, etc., seems hardly worth while. Moreover an analysis of the reports having these points in view can be made far better by those who have been in daily contact with the ill from epidemic meningitis and have learned, at first hand, to know its protean aspects and variable course. But, on the other hand, it is patent that the successful cases reported here have, almost without exception, made complete and rapid recoveries. There have been few or no long and tedious periods of convalescence, and in one instance only has a permanent defect—in this instance some impairment of hearing—remained.

Our choice of mode of introducing the antiserum into the body, namely into the spinal canal, should be justified. We were led to this manner of employment by two considerations: First, the

theoretical advantage of bringing the antiserum into direct contact with the focus of infection and inflammation, to support which we possess data based on animal experimentation; and second, the knowledge that elimination of colloids, and of crystalloids even, from the blood stream into the cerebro-spinal fluid is a slow and imperfect process in health and probably in inflamed states of the membranes also. Since there appeared to be no danger from this method of introduction of the serum, provided care was exercised, it was chosen; and the cases recorded in this paper bear testimony to its safety. In a few instances the subcutaneous has been super-added to the intradural injection, but whether any advantage is to be derived from such double injections, greater experience will have to determine. Could the subcutaneous be substituted for the intradural method there would obviously be a gain in convenience and probably in safety of general employment of the serum. We think it improbable that the results would be as good by the subcutaneous as they appear to be by the intradural method.

We possess evidence that the direct effect of the antiserum upon the diplococci present in the exudate in the cerebro-spinal membrane is to cause their rapid degeneration and an arrest of their free multiplication. This must be of some advantage to the patient. We have noted remarkable reduction in number and striking evidences of degeneration and loss of power of growth of the diplococcus, twenty-four hours after an injection of the antiserum. An exudate previously sero-purulent may be converted into a merely turbid exudate by an intradural injection of the serum.

The few cases in which the exudate has been really purulent or fibrino-purulent and present in small quantity, as judged by the few drops which could be secured on lumbar puncture, seemed not to be benefited by the serum injections. Whether such cases are very unpromising will have to be determined by wider experience with the antiserum.

Although we have discussed, briefly, the question of anaphalaxis, the cases in which several injections of serum were made, sometimes with considerable intervals between the injections, tend to show that the danger is not a very real or impending one. It can, we think, be neglected in practice for the present.

It is clear that once we accept the fact of the abortive and critically terminating cases as being caused by the antiserum, the early injection of it is to be sedulously sought. The figures, small as they are, also point to better results from the injections made in the first three days of the illness as compared with those made at a later period. And yet favorable effects have been recorded in cases treated in the fourth, fifth and eighth week of the disease. Basing a tentative deduction on the few facts of our present knowledge, we may suppose that as long as living and multiplying diplococci remain in the membranes and their fluid, the serum may be employed with hope or expectation of useful results. The serum is clearly of no avail in the treatment of symptoms resulting from chronic obstructive lesions of the membranes.

The doses of the serum employed thus far rest on an empirical basis. Whether the larger doses which have been used latterly are more efficient than the smaller ones used at first, can be determined only by a wider experience than we have yet had. In test-tube experiments degree of concentration of serum plays an important part in determining injury and disintegration of the diplococcus, since a high concentration of the serum is more effective than a low one. But the mechanism of test-tube bacteriolysis and of intradural bactericidal effect may be and doubtless are widely different. Our knowledge of the manner of *intra vitam* disposal of the diplococcus after serum injection is very defective; but an important factor is doubtless the intracellular, phagocytic digestion for which we have evidence derived from microscopical examination of the cerebro-spinal exudate. While in active stages of the infection the intracellular diplococci present sharp outlines and appear vigorous, and the extracellular microorganisms are well preserved, after the serum injection the diplococci within cells have lost sharpness of outline, stain indifferently, and strike one as degenerated, and those without cells are much reduced in numbers and staining power. Possibly it is this active and apparently accelerated intracellular digestion which prevents an increase in toxic effects following the serum injections such as might otherwise occur from the more rapidly liberated endotoxin. That the antiserum possesses certain direct antitoxic properties, which also tend to di-

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minish the dangers of endotoxin-intoxication, would seem to be indicated by its power to neutralize *intra vitam* the toxic effects of an autolysate of the diplococcus.

In order to avoid unnecessary repetition in preparing this discussion, some of the above propositions have been stated in a manner that might readily convey the impression that we regard it evident and established that the antiserum has proven its usefulness as a therapeutic agent in epidemic meningitis. The facts of our belief, at the present time, are quite otherwise. No one could be less convinced of the final fact of its value than we are. On the other hand, we believe that the data at hand warrant a wider trial of the antiserum, particularly as no other and better means of combating the disease is available. We think, however, that it is unjustifiable to employ the serum indiscriminately and without proper clinical and bacteriological controls. We shall be able, at the Rockefeller Institute, to supply a moderate amount of the antimeningitis serum for use under conditions of control which we shall prescribe.

ADDENDUM.

Since the completion of this paper reports of seventeen additional cases of epidemic meningitis in which the antiserum was employed have been received. The full histories of the cases will be published later, but summaries of them will be given here. Twelve of the cases were treated at the Municipal Hospital, Philadelphia, by Dr. Franklin Royer. A tabulation of them follows:

Case No.	Age of Patient in Years.	Day of First Serum Injection	Total Amount in c. c. of Serum Injected.	Result.
I.	3	7	180	Died.
II.	4	9	90	Recovered.
III.	18	3	90	Died.
IV.	7	3	140	Recovered.
V.	3	4	220	Died.
VI.	10	2	90	Recovered.
VII.	8	6	30	Recovered.
VIII.	22	?	95	Recovered.
IX.	13	3	150	Recovered.
X.	10	8	90	Died.
XI.	10	4	150	Recovered.
XII.	13	3	120	Recovered.

Two babies, each a year old, were treated with the antiserum at Mt. Sinai Hospital, New York, by Dr. Heiman. One received the first injection of serum on the twenty-third day, 60 cubic centimeters were injected in all; it died. The other was injected on the fifteenth day, received 35 cubic centimeters of the serum and recovered.

Two children were treated with the serum at the Babies Hospital, New York, by Dr. Holt. One is eleven months old, was injected first on the forty-ninth day of illness and will probably die. The other is eighteen months old, was injected first on the twenty-third day of illness and is now convalescent.

An additional case, not included in the first series of cases, was treated at the Akron City Hospital. The child was six years old, was injected first on the sixth day of illness, received in all 160 cubic centimeters of antiserum and recovered.

Zur Gewinnung des Isoleucins aus Eiweißspaltungsprodukten.

Von

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(From the Rockefeller Institute of Medical Research. New York.)

(Eingegangen am 29. Januar 1908.)

Bei der Darstellung des Isoleucins aus den Spaltungsprodukten der Proteine sind mehrere Forscher auf fast unüberwindliche Schwierigkeiten gestoßen, die dadurch verursacht waren, daß es kein sicheres Verfahren zur Trennung des Leucins von Valin gab. — Nun ist es einem von uns gelungen, diese Trennung leicht durch Fällern des Leucins mittels neutralen Bleiacetats und Ammoniaks zu bewirken. Nach dem Befreien dieses Niederschlages von Blei erhält man gewöhnlich ein Präparat, das ohne Umkrystallisation die Zusammensetzung des reinen Leucins besitzt. — Es war aber noch unentschieden, welche Form des Leucins hier vorlag, und es wurde ein Versuch vorgenommen, die nach diesem Verfahren dargestellte Substanz nach den Angaben von Ehrlich aufzuteilen. — Tatsächlich kann man sich ohne Schwierigkeiten davon überzeugen, daß ein Gemisch von zwei Isomeren vorliegt.

Einige Kilo käuflichen Caseins wurden mit 33 % Schwefelsäure gespalten von der überschüssigen Säure befreit und eingedampft. — Man stieß dabei auf große Schwierigkeiten, das Leucin frei von Tyrosin zu gewinnen. — (Wie bekannt, kommt das nicht bei Hydrolyse mittels Salzsäure vor). Das Tyrosin vollständig zu entfernen wurde nur durch Behandeln mit Bromwasser ermöglicht. — Die Bromverbindungen wurden mittels Amylalkohol ausgeschüttelt, und das Gemisch von Leucin und Valin schied sich beim Eindampfen der wässrigen Flüssigkeit aus. — Aus der Lösung dieser Substanzen wurde das Rohleucin durch Bleiacetatlösung und Ammoniak gefällt. — Vom Blei befreit, besaß die Substanz die folgende Zusammensetzung:

0,1355 g der Substanz gaben 0,2743 g CO₂ und 0,1255 g H₂O für C₆H₁₃NO₂

0,0355 g Berechnet	Gefunden
C = 54,96	55,17 %
H = 9,92	10,28 %

Diese Substanz wurde dann in Wasser gelöst, mit Kupfercarbonat gekocht, abfiltriert und zur Trockne bei vermindertem Drucke eingedampft. Der Rückstand wurde noch einmal mit absolutem Alkohol bei demselben Druck eingedampft, um Spuren vom Wasser zu entfernen, dann mit absolutem, wasserfreiem Methylalkohol ausgekocht, das Filtrat von Methylalkohol und Kupfer befreit und das Isoleucin durch Auskrystallisieren aus Wasser erhalten.

Es besaß die folgende Zusammensetzung:

0,1529 g der Substanz gaben 0,3063 g CO₂ und 0,1387 g H₂O für C₆H₁₃NO₂

Berechnet	Gefunden
C = 54,96	54,64
H = 9,92	10,08

0,6413 g der Substanz wurden in 15 ccm 20% Salzsäure gelöst. Das Gesamtgewicht der Lösung betrug 16,5252 g. Sie drehte bei 20° im Na-Licht in einem 1,894 Dec.-Rohr 2,77° nach rechts. Sp. Gew. 1,1

$$[\alpha]_D^{20} = + 34,26^\circ + (0,1)$$

Es lag also Isoleucin vor.

Die Kupferverbindung, die sich in Methylalkohol nicht öste, wurde vom Kupfer befreit, und die resultierende Substanz erwies sich beim Umkrystallisieren aus Wasser als das gewöhnliche Leucin.

0,6442 g der Substanz wurden in 15 ccm 20% Salzsäure gelöst, das Gewicht betrug 15,6598 g. Sp. Gew. 1,1. Die Lösung drehte bei 20° C im Na-Licht in einem 1,894 Dec.-Rohr 1,54° nach rechts.

$$[\alpha]_D^{20} = + 18,00 + (0,1)$$

Dieses stimmt mit den Angaben anderer Forscher für Leucin gut überein.

Zur Herkunft des Cytosins bei der Hydrolyse der tierischen Nucleinsäuren.

Von

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(From the Rockefeller Institute of Medical Research and from the Chemical Department of the New York University and Bellevue Medical College, New York.)

(Eingegangen am 25. Februar 1908.)

Der Ursprung des bei der Nucleinsäurenhydrolyse resultierenden Cytosins wurde von Burian seit mehreren Jahren in Frage gestellt. Die Erfahrungen bei der Hydrolyse verschiedener Nucleinsäuren scheinen zwar nicht mit den Ansichten von Burian in Einklang zu stehen, daß Cytosin ein sekundäres Produkt sei. Die größere Ausbeute und die leichte Gewinnbarkeit der Substanz aus den pflanzlichen im Vergleich mit den tierischen Organen scheint dieser Theorie zu widersprechen. Von mehreren Nucleinsäuren tierischen Ursprunges, die wir untersuchten, verhielt sich nur eine aus den Eiern des Schellfisches wie die pflanzlichen. In dieser Säure wie auch in den pflanzlichen Nucleinsäuren ist allem Anscheine nach das Uracil kein sekundäres Produkt.

Nun aber verhält sich bei der Hydrolyse von tierischen Nucleinsäuren das Cytosin von dem Thymin verschieden. Während dieses in seiner Ausbeute von den Bedingungen der Hydrolyse wenig abhängig und schon durch einfaches Erhitzen der Nucleinsäure mit Wasser im Autoklaven auf 150 bis 175° C zu erhalten ist, hängt die Ausbeute an der andern Verbindung von scheinbar unwichtigen Umständen wesentlich ab. Gerade deswegen schien seit längerer Zeit eine eingehende Untersuchung über den Ursprung des Cytosins der Nucleinsäuren

diringend erforderlich zu sein. Wir haben schon mehrere Versuche im vergangenen Winter vor dem Erscheinen der letzten Arbeit von Burian angestellt. Die Arbeit bestand hauptsächlich in einem Versuche, die Bedingungen zu studieren, bei welchen die Ausbeute an Cytosin die vorteilhafteste war, und zu erforschen, ob die für die Ausbeute günstigsten Einflüsse auch diejenigen waren, welche zu der Entstehung der Substanz aus Purinbasen führten. Da Burian¹⁾ den Gedanken ausgesprochen hat, daß die bei der fermentativen Spaltung vorkommende Pyrimidine sekundärer Natur seien, so wurde ein Versuch auch auf die Entscheidung dieser Frage gerichtet.²⁾

Schon in einer früheren Mitteilung³⁾ über die Nucleinsäure des Maifisches wurde die Beobachtung erwähnt, daß die Ausbeute des Cytosins viel günstiger war, wenn das Kupfersalz zur Anwendung kam, als bei Verarbeitung von Präparaten, die mit Alkohol gefällt waren. Dieses konnte nur entweder davon abhängen, daß bei Alkoholfällung Substanzen mitgerissen wurden, die auf die Ausbeute störend wirken, oder daß die Anwesenheit von Kupfer die Ausbeute begünstigte. Es wurden deswegen 3 Präparate gespalten; Präparat 1 war aus der Lösung der Säure in Eisessig mit Alkohol gefällt, Präparat 2 mit Kupferchlorid, Präparat 3 war aus einer wässerigen Lösung des Präparates 1 mit Salzsäure gefällt.

Die Ausbeuten an Cytosin waren die folgenden:

Präparat 1: 30,0 der Substanz 4 Stunden im Autoklaven mit 125,0 ccm 25% Schwefelsäure erhitzt. Die Ausbeute betrug 1,0 g.

Präparat 2: 25,0 der Substanz wie oben behandelt. Die Ausbeute betrug 4,0.

¹⁾ Ergebnisse der Physiologie 1, 98, 1904, und Zeitschr. f. physiol. Chem. 51, 438, 1907.

²⁾ Bald nach dem Erscheinen der Arbeit von Burian hatte einer von uns eine kurze Notiz über die damals schon ausgeführten Versuche an Herrn Professor A. Kossel zur Publikation in der Zeitschr. f. physiol. Chem. gesandt, in welcher erwähnt wurde, daß weitere Versuche im Wintersemester fortgesetzt werden würden. Herr Professor Kossel hat die Notiz zu veröffentlichen nicht für möglich gefunden. Nun sind unterdessen die Experimente von Burian wiederholt und in einem der letzten Hefte der Zeitschr. f. physiol. Chem. veröffentlicht worden.

³⁾ Zeitschr. f. physiol. Chem. 50, 1, 1906.

Präparat 3: 25,0 der Substanz wie in den zwei anderen Experimenten behandelt. Die Ausbeute betrug 3,8 g.

Aus dieser Erfahrung darf man schließen, daß bei der Alkoholfällung Substanzen mitgerissen werden, welche die Ausbeute an Cytosin stören, entweder weil sie ihre Abspaltung verhindern oder weil sie mit der Verbindung Kondensationsprodukte bilden. Es war aber bei diesen Versuchen auffallend, daß, wie auch bei den früher mitgeteilten, in dem Falle, wo die Ausbeute an Cytosin gering war, noch viel Adenin gewonnen werden konnte. Dieses konnte auf einen Zusammenhang der beiden Erscheinungen hindeuten. Zur Entscheidung dieser Möglichkeit wurde versucht, ein Gemisch von Adenin und Glucose sowie von Guanin und Glucose mit und ohne Anwesenheit von Kupfer im Autoklaven zu erhitzen. Die Resultate waren die folgenden:

Experiment 1: 5,0 Guanin und 5,0 Glucose im Autoklaven mit 300 ccm 33% Schwefelsäure 3 Stunden auf 150° C erhitzt.

Das unveränderte Adenin wurde mit Silbernitrat gefällt und die Pyrimidinbasen nach Kossel-Jones darzustellen versucht. Es konnte aber kein Cytosin in der Silber-Baryt-Fraktion nachgewiesen werden.

Experiment 2: 5,0 der Substanz mit 5,0 Glucose und 5,0 Kupfersulfat wie im ersten Experiment behandelt. Auch hier konnte kein Cytosin aufgefunden werden.

Experiment 3: 7,5 Guanin wurde mit 8,0 Glucose mit 400 ccm 40% Schwefelsäure mit Rückflußkühler 12 Stunden erhitzt und genau die Angaben von Burian befolgt; auch da war kein Cytosin nachweisbar.

Experiment 4: 5,0 Adeninsulfat mit 5,0 Glucose im Autoklaven mit 33% Schwefelsäure für 3 Stunden erhitzt. Es konnte kein Cytosin aufgefunden werden.

Experiment 5: 4,0 Adeninsulfat mit 5,0 Glucose und 5,0 Kupfersulfat und 250 ccm 33% Schwefelsäure 3 Stunden im Autoklaven auf 180° C erhitzt. Es konnte kein Cytosin aufgefunden werden.

Alle diese Versuche konnten also keine Anhaltspunkte für eine Abstammung des Cytosins aus Purinen liefern. Diesen entgegen standen aber die positiven Befunde von Burian. Es schien

deshalb notwendig, diese Frage noch eingehender zu erforschen. Nun könnte man denken, daß bei der Hydrolyse der Nucleinsäuren die Bedingungen zur Bildung von Pyrimidinbasen aus Purinen am günstigsten sind. Sollte die Sache wirklich so sein, dann dürfte man erwarten, daß diejenigen Säuren, welche am reichsten an Purinbasen sind, auch die besten Ausbeuten an Cytosin liefern würden. Wir versuchten deshalb den Gehalt der Säure an Purinbasen zu ändern und sie dann der Hydrolyse zu unterwerfen. Solche Versuche hat schon früher einer von uns mitgeteilt. Aber die Resultate waren nicht ganz eindeutig, wie Burian mit Recht bemerkt hat.

Nun sind aber die Schwierigkeiten, solche Präparate herzustellen, so groß, daß auch dieses Mal die Versuche nicht ganz befriedigend sind.

Schon in einer der früheren Arbeiten¹⁾ war bemerkt, daß sich bei der unvollständigen Hydrolyse der Nucleinsäuren ein löslicher und ein unlöslicher Teil bilden. Der lösliche Teil enthält, wie dann ausgefunden war, Purine und eine komplizierte Substanz, welche bei weiterer Hydrolyse Thymin und Cytosin lieferte. Die Existenz der komplizierten Substanz wurde nur auf indirektem Wege bewiesen, und deswegen war die Möglichkeit des Purinursprunges von Cytosin nicht ganz ausgeschlossen. Nun haben wir jetzt versucht, die Purinbasen zu entfernen, bevor die lösliche Fraktion der weiteren Hydrolyse unterworfen war.

Man hoffte dieses durch Fällen der Purinbasen mittels Phosphorwolframsäure zu erreichen, da nach einer Angabe von A. Kossel die Nucleinsäuren im Überschuß von diesem Reagens unlöslich sind. Es wurde auf folgende Weise verfahren: Etwa 80,0 g Nucleinsäure wurden im Autoklaven mit 400 ccm Wasser und 8,0 g Barythydrat (um die sich bildende Phosphorsäure teilweise zu neutralisieren) auf 180° C 8 Stunden lang erhitzt. Wie zu erwarten war, ist der größte Teil der Säure in Lösung gegangen. Die Flüssigkeit reagierte sauer auf Lackmus. Die Lösung wurde vom Rückstande abfiltriert und in 4 Teile geteilt.

Teil 1 wurde ohne weitere Hydrolyse auf Purin und Pyrimidinbasen untersucht.

¹⁾ Zeitschr. f. physiol. Chem. 45, 370, 1905.

Teil 2 wurde mit Phosphorwolframsäure behandelt, der Niederschlag auf Purinbasen untersucht und das Filtrat von Phosphorwolframsäure befreit, eingedampft und mit einer 25%₀ Lösung von Schwefelsäure im Autoklaven bei 175° C hydrolysiert.

Teil 3 wurde zuerst mit 2%₀ Phosphorsäure im Autoklaven 6 Stunden auf 100° C erhitzt, dann mit einer 10%₀igen Phosphorwolframsäure behandelt, um die Purinbasen niederzuschlagen. Das Filtrat und der Niederschlag wurden wie im vergangenen Experiment behandelt.

Teil 4 wurde direkt mit etwa 25%₀ Schwefelsäure hydrolysiert.

Wir hatten geglaubt, daß durch die Phosphorwolframsäure hauptsächlich die Purinbasen und das Cytosin, welches schon beim Erhitzen mit Wasser sich bildete, entfernt werden. Im Filtrate sollten die intermediären Produkte vorhanden sein, welche nach weiterer Hydrolyse die Pyrimidinbasen bilden sollten. Im Teile 3 sollten mehr Purinbasen abgespalten werden und deshalb die intermediären Produkte ärmer an diesen Basen sein; und sollten die Purinbasen an der Bildung von Cytosin teilnehmen, so dürfte man in Teil 4, wo diese Basen nicht entfernt waren, die größte Ausbeute an Cytosin erwarten, sie sollte nicht so groß in Teil 2 und noch kleiner als in Teil 3 sein.

Im Experiment 4 war die Ausbeute wirklich die größte, aber in Versuch 2 und 3 konnte man keinen Unterschied zugunsten des zweiten Experimentes merken. Die Ausbeute an Cytosinpicrat betrug in Experiment 4 etwa 1,0 g, in Experiment 2 Spuren und in Experiment 3 Spuren. In Experiment 1 konnte (Verfahren von Kossel-Jones) von den Pyrimidinbasen nur Thymin nachgewiesen werden. Die Ausbeute an Thymin war in allen Experimenten fast die gleiche.

Also nach diesen Versuchen dürfte man annehmen, daß beim Erhitzen der Nucleinsäure mit Wasser auf 175° C sich kein Cytosin bildete, daß die dabei entstehenden Produkte bei weiterer Hydrolyse Cytosin bildeten, aber über den Anteil, welchen die Purinbasen an diesem Vorgange nehmen, kann man aus dem vorliegenden Versuche nichts schließen.

Es wurde dann versucht, die Purinbasen durch Silberfällung zu entfernen und das Filtrat von diesem Niederschlage auf die Fähigkeit zur Cytosinbildung zu prüfen.

20,0 lufttrockene Nucleinsäure wurden im Ölbad mit 400 ccm

2%igen Schwefelsäure 4 Stunden auf 150° C erhitzt. Es entstand dabei eine braune Lösung und eine kaum merkbare Quantität von humusartigen Substanzen. Das Filtrat wurde mit Silber-sulfatlösung gefällt und das Filtrat vom Silberniederschlage vom Silber mittels Schwefelwasserstoff befreit, bei vermindertem Druck bis auf 50 ccm eingedämpft und im Autoklaven 4 Stunden bei 150 bis 175° C erhitzt. Die resultierende Flüssigkeit in üblicher Weise auf Thymin und Cytosin untersucht. Die Ausbeute an Cytosin-pikrat, einmal aus Wasser umkrystallisiert, betrug 1,0 g.

Die Zusammensetzung des Pikrates war die folgende:

0,1024 g der Substanz ergaben bei Verbrennung 22,0 ccm Stickstoff bei 20° C und 757 mm;

Berechnet für $C_4H_5N_3O \cdot C_6H_2(NO_2)_3OH$:	Gefunden:
N = 44,70%	44,45%

Im Vergleiche mit der oben angegebenen Ausbeute aus Präparate 2 und 3 sollte man etwa 3,0 g der Substanz erhalten. Man darf aber durchaus nicht aus diesem Versuche schließen, daß die kleinere Ausbeute an Cytosin durch die Entfernung der Purinbasen verursacht war. Wir konnten uns nämlich überzeugen, daß mit dem Silbersulfat außer den Purinbasen noch andere kompliziertere Substanzen mitgerissen wurden. Über die Natur dieser Substanzen wird in einer anderen Mitteilung eingehend berichtet werden. Doch sind die Resultate dieses Versuches nicht ganz eindeutig. Nun wurde versucht, den Einfluß der Zugabe von Purinbasen zu der Nucleinsäure auf die Ausbeute an Cytosin bei der Hydrolyse zu ermitteln.

25,0 g Nucleinsäure wurden mit 5,0 g Guanin und 3,0 g Adeninsulfat im Autoklaven mit Schwefelsäure von 25% 4 Stunden lang auf 150 bis 175° C erhitzt und in üblicher Weise auf Cytosin untersucht. Die Ausbeute betrug 4 g, also zeigte sich kein günstiger Einfluß des zugesetzten Guanins und Adenins.

Also auch diese Versuche konnten keinen Beweis dafür bringen, daß das Cytosin aus den Purinbasen stammt.

Es hat einer von uns schon vor mehreren Jahren bei der Autolyse von Pankreasdrüsen das Vorkommen von Uracil¹⁾ beobachtet und dann diesen Befund als einen Beweis für den

¹⁾ P. A. Levene, Zeitschr. f. physiol. Chem. 32, 541, 1901.

Anteil dieser Substanz im Aufbau des Nucleinsäuremoleküles betrachtet. Nun bemerkt Burian mit Recht, daß noch der Beweis fehle, daß Cytosin sich bei der Autolyse der Organe nicht aus Purinbasen bildete. Nun wurden 3,0 g Guanin mit 10,0 g Glucose und 50,0 g Milzgewebe in 500 ccm 0,2% Essigsäurelösung aufgenommen, mit Toluol 2 Monate lang im Thermostaten gelassen und dann nach dem Kossel-Jonesschen Verfahren auf Cytosin und Uracil untersucht. Es war aber unmöglich, diese Substanzen zu gewinnen. Zu allen Versuchen ist Milznucleinsäure gebraucht worden und die Identifizierung des Cytosins als Pikrat erfolgt.

AN INQUIRY INTO SOME MECHANICAL FACTORS IN
THE PRODUCTION OF LYMPHOCYTOSIS.

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The paper that follows deals with an attempt to separate out, and study, some of the factors which produce the clinical feature of lymphocytosis. The truth of Ehrlich's (1) doctrine that an absolute lymphocytosis is due, apart from changes in the productive activity of the lymphoid tissue, to a flushing out of the cells through increase in lymph-flow, though supported by clinical evidence, has never been proved. Indeed, there have been few attempts to come directly by experiment to the forces determining lymphocytosis; this, too, despite manifold labours to plot the fluctuation of the blood-content in lymphocytes caused by divers physiological and pathological conditions.

For the experiments here detailed the cell-output by way of the thoracic duct was utilized. Of late this path to the blood for lymphocytes has been held to be of comparatively little importance. The recognition of lymphoid centers in the bone-marrow, the study of the abundant lymphadenoid tissue of the digestive tract, the observations for a direct passage of the lymphocytes into the blood-vessels, and the realization that the lymph has important functions of its own, have tended to this conclusion, as have the many assertions that the lymph of the thoracic duct carries few lymphocytes in comparison with the blood's needs. Nowadays, as Delamere (2) says, we hold that "the lymphocytes are the casual guests of the lymph." They are supposed to be formed in the lymph-glands and the lymphadenoid tissues in general, the spleen, and the bone-marrow, with direct entrance through the vessel-walls as a frequent way by which they reach the blood.

¹ Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication November 18, 1907.

Nevertheless, recent experimental evidence points to the thoracic duct as the chief way to the circulation for the lymphocyte. Biedl and v. Decastello (3), working on dogs, produced fistula of the thoracic duct, and found that the lymphocytes in the blood decreased between 18 per cent. and 62 per cent. in absolute number; suspecting accessory channels, they carefully ligated the lymphatics on both sides of the neck, and obtained in the one animal so treated a diminution in the lymphocytes of 79 per cent. Selinoff (4), in a study of the blood of 18 dogs with fistula of the thoracic duct, noted an even more marked decrease. Thus, for example, in two of his cases there were, respectively, 1,800 and 2,000 lymphocytes per cubic millimeter in the blood just prior to operation, and on the fifth day thereafter, in the first case, only 100 such cells, and on the seventh day, in the second case, only 200 such cells per cubic millimeter. He made certain by controls that these results could not be laid to the effects of the operation itself. Crescenzi (5) observed the blood after splenectomy and the establishment of a fistula of the thoracic duct in the same animals. He obtained a decrease in the lymphocytes of from four fifths to ten elevenths of their number. Parodi (6) following Crescenzi and Selinoff, came to the conclusion that, in dogs, fistula of the thoracic duct, with or without splenectomy, brings about a diminution in the quantity of lymphocytes in circulation. Unfortunately he omits the figures supporting this. Yet those cited above seem convincing when one considers the direct anastomoses known to exist between the lymphatics and the blood-vessels (Lippi, Boddaert, Leaf (7)), and the undoubted migration of some lymph-cells directly through tissues into the blood-stream. True, the diminution that the figures represent is transitory; but this only emphasizes the presence of a compensatory mechanism that must mask, to an extent, the full effect of the fistula.

It has been objected that the number of cells furnished to the blood through the thoracic duct is quite inadequate to maintain the percentage of lymphocytes seen. But the important element in such calculations,—the term of existence in the circulation of the individual cell,—is not known. The number coursing through the thoracic duct (from 2,000 to 7,000 in the cubic millimeter of dog's

lymph—Winternitz (8)) may be quite adequate, as Biedl and v. Decastello are at pains to show, for the needs of the circulation.

Whether or not the thoracic duct furnish the majority of the lymphocytes to the blood, as above indicated, the system of which it is the outlet,—a more or less completely “closed” system with one principal duct,—is the part of the hæmatopoietic apparatus most accessible to direct investigation as regards variation in cell-output.

The cell-content of the lymph has been comparatively little observed, and this mostly before the discovery of the bone-marrow as a blood-forming organ, and hence before the study of the blood-cells in its modern sense. Following Virchow's (9) demonstration of the identity of the “small mononuclear” with certain elements found in lymph-glands, several observers showed these cells to be more numerous in the lymph coursing from a gland than in that coming to it (Heydfelder, Brucke, Frey (10)). Löwit (11), counting the elements from the thoracic duct of the rabbit, obtained a great increase in them by the administration of substances causing blood-leucocytosis,—a result which has since drawn some criticism. Winternitz took lymph from the vessels of the dog's thigh, following the injection of turpentine into the corresponding foot, and came to the conclusion that with inflammation of a part the cell-content of the lymph coming from it is increased, and the majority of the cells becomes one of polymorphonuclear neutrophiles. Goodall and Paton (12), during an investigation on digestive leukocytosis, made counts from several points in the lymphatic system, but with very irregular results, except for evidence that pointed to a sedimentation of the cells in the receptaculum chyli. Recently Forgeot (13) has examined the lymph of ruminants as it escaped from a thoracic duct fistula, with no constant findings, however, beyond that of a greater cell-content in the fluid from young individuals. There have been no adequate researches on the cell-content of the lymph under varying physiological conditions. Yet, needless to say, the quantity of cells in the lymph represents one side of the activity of the lymphadenoid tissue; and variations in this quantity, in addition to their value as an index to the state of that tissue, have a bearing on clinical lymphocytosis and lymphopænia, and, ultimately, on the meaning of the lymphocyte.

To obtain a count that represents the average number of elements in the lymph flowing at a certain time, one should obtain a thorough mixture of a considerable quantity of it. For, by reason of the inconstancy of lymph-flow, as tending to a sedimentation of cells, and the anatomical arrangement of the lymphatics, which prevents the mingling of successive portions of the fluid, it follows that the individual drops, as they come from the vessel, must differ much in cell-quantity. Nevertheless one finds that of the few authors who have interested themselves in the cell-content of the lymph, practically all have taken their counts from the single drop,—which accounts for much of the irregularity in their results. Those above cited did this, except Forgeot who, in his work on ruminants, utilized one quarter of a cubic centimeter,—an extremely small quantity, considering the large size of the animals on which he experimented. Dastre, Henri, and Stodel (14), in an investigation of the effect of peptone on the cells, allowed the fluid to collect in the ligated end of the thoracic duct, or in the subclavian vein, there mixing it “by light, inconstant pressure.” But, in addition to awkwardness, this measure gives the opportunity for large error in successive counts. I have employed, for the work here reported, a means whereby could be utilized a quantity of lymph sufficient to insure a result representing the average cell-content of the lymph at that time. Since dogs were the animals used, and the thoracic duct the point of collection, several cubic centimeters were deemed necessary for each test, owing to the large lymph-flow (64 c.c. per kilo per diem, or, in a dog of 18 kilo, 4 c.c. in every 5 minutes,—Heidenhain (15)). The following technic was adopted:

Three cubic centimeters of lymph are allowed to flow into a tube that contains an equal quantity of a 4 per cent. solution of sodium citrate in 0.8 per cent. salt solution,—a mixture suggested by Wright (16) for the preservation of blood unclotted and with its elements intact.² The tubes for this purpose are 9 mm. in bore, and are graduated accurately to 3 c.c. and 6 c.c. By reason of the

² Wright recommends the use of 1 part of the sodium citrate solution to 5 parts of human blood. So little of the solution will not keep the dog's lymph from clotting. A mixture of the two in equal parts is just sufficient to serve the purpose.

narrow caliber, it is easy to control an error in volume to within the limits of a single drop, and, with care in the preliminary introduction of the sodium citrate solution, to confine this error almost entirely to the amount of lymph added. But let us suppose in the combined bulk of 6 c.c. (or about 90 drops) the largest tolerable error,—that of one drop in the quantity of sodium citrate solution added, and of one drop in the total mixture. The extremes here possible are, 44 drops sodium citrate solution to 47 drops lymph, and 46 drops sodium citrate solution to 43 drops lymph; or $47/91$ of lymph in the first and $43/89$ in the second mixture, a variation from the supposed ratio ($45/90$) of slightly over 3 per cent., or an error of 150 cells in a count of 5,000. This may be neglected.

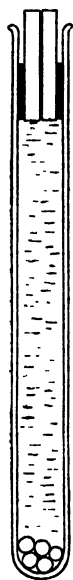


FIG. 1.

The lymph as it falls from the cannula into the sodium citrate solution is mixed with this by means of a fine wire, and, when the 3 cubic centimeters have been obtained, a few glass beads are introduced to aid in the distribution of the cells on shaking, and the tube stoppered preliminary to this. For stoppering a piece of glass rod with a flange of rubber is used, a capillary opening through the center of the rod permitting the escape of air so that the stopper may be pushed flush with the fluid. (*Vide* sketch in cross-section.) The closed tube is shaken for 5 minutes; a portion of its contents drawn into a "mélangeur" with 1/100 its bulk of a saturated aqueous solution of methyl violet (5B): this in turn shaken for three minutes, and a count made as for blood. The lymph is thus counted in about 1/2 concentration (99 parts lymph to 101 parts diluting fluid). Leukocytes take the violet stain, whereas erythrocytes do not.

To test the method counts were made at intervals from the same tube of lymph-sodium-citrate mixture.

Thus at the end of seven hours the leukocyte-count coincides, practically, with that first taken. The cells are undergoing degenerative changes at that time, yet may be easily enumerated. But it is necessary, for this, that the tube be agitated at least once per hour. Otherwise, the cells sediment, cohere, and cannot be easily distributed again.

Dog.	Tube No.	Time between Counts.	R. b. c. per cmm. Lymph.		W. b. c. per cmm. Lymph.	
			At First Count.	At Second Count.	At First Count.	At Second Count.
Jl	V	2 hours	not tested		3,880	4,240
El	XII	2½ hours	440	440	11,740	12,360
Nl	VII	2½ "	39,840	22,240	8,980	10,080
Ol	VI	3 hours	3,940	1,810	not tested	
MI	V	3½ hours	5,080	3,720	3,300	3,660
Ll	II	4 hours	18,560	10,960	11,920	11,180
Gl	III	5 "	not tested		13,120	13,440
Kl	I	5 "	1,800	1,000	2,400	2,120
Ol	I	5 "	not tested		6,760	6,900
Pl	I	5 "	3,120	1,180	2,760	2,800
Jl	III	7 "	14,720	12,800	4,060	3,800
El	X	18 "	not tested		7,040	6,440

It is different with the erythrocytes. These seem to disappear rapidly in the mixing fluid, notwithstanding the fact that, to judge from the unchanged or slightly crenated shape of such corpuscles as remain, and the absence of shadows, this destruction is not due to osmotic changes. Since the lymph of nearly all dogs contains red cells, an idea of their quantity, granting them to be due to blood-contamination, is important in work having to do with the white cells of the lymph, since it furnishes an index to the number of leukocytes also brought in from the blood. But if, on the meeting of the lymph with the sodium citrate solution, many of the red cells go immediately to pieces, this index is destroyed; and one might have in the specimen many white cells from the blood without trace of the contamination, so far as red cells are concerned. This possible source of error was tested for as follows:

Dog Cl.—Blood taken during the experiment had 8,600,000 r.b.c. and 13,800 w.b.c. per cmm., of which last 70 per cent. proved to be polymorphonuclear neutrophils, giving thus 9,660 such cells in the cmm. of blood, or about 1 to every 890 r.b.c. The lymph at this time contained on count from the sodium citrate mixture 1,545 w.b.c. and 5,685 r.b.c. per cmm. Calculating from the ratio existing in the blood, one should have 6 polymorphonuclear neutrophils introduced with these red cells. A differential count of the lymph obtained at the time will test this supposition, since the normal lymph of the dog contains extremely few polymorphonuclear neutrophils of its own (Delamere, Biedl and v. Decastello). As a matter of fact, in this instance the lymph showed out of 384 cells counted 2 polymorphonuclear neutrophils, or 8 in the 1,545 w.b.c. of a cmm. So the number of red cells found in the lymph seemed to be practically that introduced from the blood. Several controls of this type gave the same result.

Despite this proof that the sodium citrate solution is, for *prompt* enumeration, a medium wherewith can be obtained an approximate estimate of the red cells, no lymph was admitted for the white count, of which the erythrocyte content was large enough to suggest that the accompanying leukocyte contamination might influence appreciably the results. The number of polymorphonuclear neutrophiles found in the lymphs used formed, as above shown, an additional indication of the amount of this contamination.

In the work here detailed the lymph's content in white cells is alone dealt with.

The effects of muscular exertion (struggle) on the cell-content were first observed. Adult dogs were employed. They were given 0.5 centigramme of morphia sulphate per kilo of body-weight (Nolf (17)) 1 hour before the operation, and chloroform when necessary during it. For from 24 to 48 hours prior to the operation no food was allowed the animals, though they were provided with water. The thoracic duct was bared in the usual way, a cannula introduced into it just above its entrance to the vein, and the entrance tied off together with such lymphatics from the neck as joined the thoracic duct, with the result that the fluid brought by the thoracic duct proper was alone collected. During this procedure very little blood, at most 3 to 4 cubic centimeters, was lost. The cannula used was of narrow bore, as recommended by Nolf, since the rapid flow through such a tube allows little opportunity for clotting. Nevertheless, in about half the experiments a delicate clot formed within the cannula in the course of some minutes, so that the occasional use of a fine hooked wire was required to keep the bore clean. No tubes of lymph were counted in which the least clotting appeared, nor were any used regarding which it seemed possible that clots in the cannula might have altered the gross cell-number. The presence or absence of clotting is mentioned in the report of the individual experiments.

It was first necessary to observe the variations in the lymph's cell-content under the circumstances above outlined and with the animal quiet, since these circumstances do not imply an absence of changes that might affect the cell-content. The shunting of the lymph from the body, following the opening of the thoracic duct,

produces marked alterations in the body-fluids ~~(the blood,~~ for example, concentrating, the lymph becoming less in amount and of different character). This might affect the lymph's cell-content. Furthermore, as the experiment progresses, the effect of the morphia wears off and chloroform must be pressed into service. Other unavoidable changes might be cited. The behavior of the cell-content under these influences must be reckoned with before one can proceed.

Accordingly, in animals carefully anæsthetized to a state of quiet, though not of complete muscular relaxation, a lymph-fistula was established, and specimens of lymph collected at short intervals during the next several hours. The time required to obtain each portion of three cubic centimeters was carefully noted as indicating the rate of lymph-flow at that period. Full records were also kept of all restlessness of the animal, of the incidents of anæsthesia, etc. When important, these are included in the description of the experiments.

Experiment I.—Mongrel collie; male; wt. 13 kilo. The animal was given no food for 48 hours previous to experiment. Throughout the time of lymph-collection it was quiet, except for occasional tremors in the limbs. Lymph very slightly opalescent; no clotting noted in cannula or tubes. Seven tubes were taken, and immediate estimate made of their content in white cells.

The dog at autopsy proved to have been sound, except for a chronic thickening of one segment of the tricuspid valve; no evidences of functional insufficiency of this valve.

The results are best expressed in the form of a chart. (Chart 1.)

Of the three curves on this chart one represents the rate of lymph-flow, a second the number of cells per cubic millimeter of lymph, and the third (which is the resultant of these two) the total cell-output in a given period.

It will be observed that throughout the course of the experiment the lymph-flow gradually but steadily lessened in rapidity, and hence in amount voided. This is, of course, no new finding (Lassar (18), Heidenhain and others). The number of cells per cubic centimeter of lymph, the "cell-concentration," as it will henceforth be termed, remained nearly constant, sinking slightly at the last count. It follows from these findings that the total cell-output underwent a marked gradual diminution.

To test these results two similar experiments were done.

Experiment II.—Collie; male; wt. 23 kilo. The animal was given no food for 48 hours previous to operation. Lymph began to escape from the thoracic duct (which had been ligated 1 minute before opening) 15 minutes prior to the collection of the first tube for cell-estimation. It was very slightly opalescent; no clotting was observed. Counts taken in each case immediately after collection. Eight tubes were obtained at half-hour intervals. Throughout, the animal was absolutely quiet. (See Chart 2.)

Dog killed and autopsied; it proved to have been quite healthy.

Here the same lessening of the lymph-flow is noted. The cell-concentration, markedly greater than in Experiment I, fluctuated

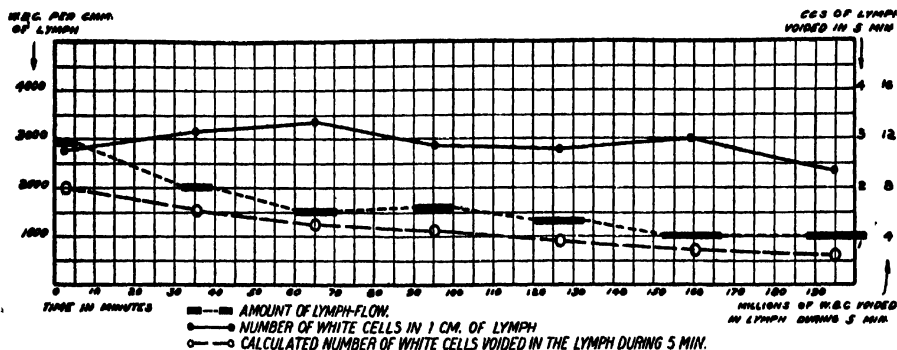


CHART I.

CHART I. The height above the base-line of the curve representing amount of lymph-flow indicates the number of cubic centimeters voided through the thoracic duct in a given time; and the black rectangles show the period required to collect the three cubic centimeters of lymph in each specimen. Thus the curve depicts in two ways the rapidity of lymph-flow.

proportionately, but in general remained constant during the first $2\frac{1}{2}$ hours, after which it rose abruptly. The cell-output fell till toward the close of the experiment, when it regained nearly its former level.

Experiment III.—Skye-terrier; male; wt. 11 kilo. The fast before operation was of 24 hours duration, yet the lymph was quite chyliiform throughout the period of observation. Clotting in the cannula necessitated several times the use of the hooked wire to avert blocking of the lymph-flow. The thoracic duct was ligated one half hour, and opened 15 minutes before the collection of the first tube for cell-estimation. The contents of the tubes were submitted to count in the order of their collection, but not till 3 to 4 hours after it, that is to say

at the close of the experiment proper. Seven tubes were collected at intervals in a period of 240 minutes. (See Chart 3.)

Animal normal, to judge from findings at autopsy.

A gradual drop in the rapidity of the lymph-flow occurred, similar to that in the other experiments, except for the presence of two transient fluctuations, apparently traceable to respiratory changes. The cell-concentration remained practically constant throughout the four hours, at the end of which it differed by only 100 cells per

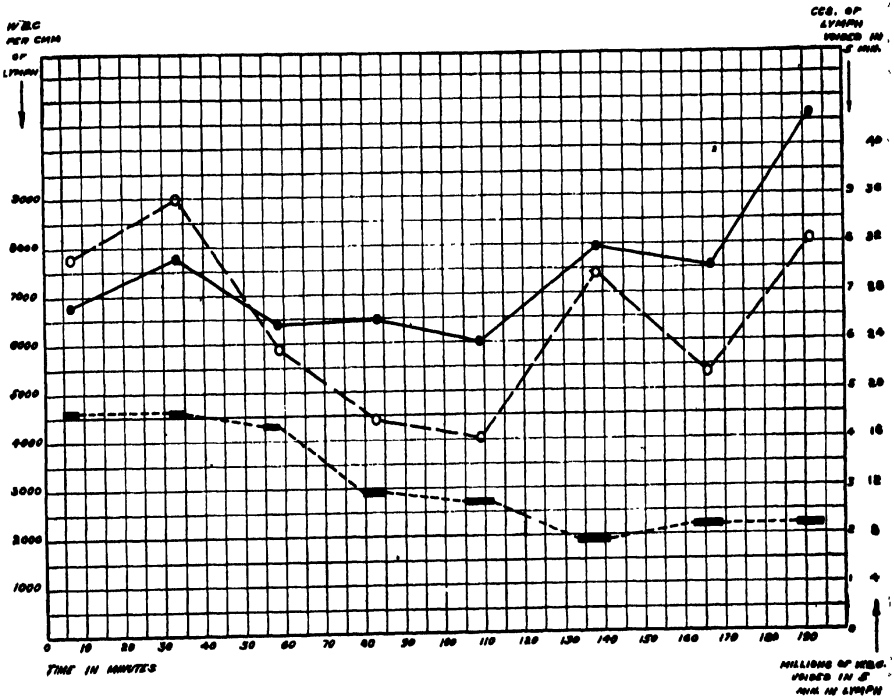


CHART 2.

cubic millimeter from that at the beginning. The total cell-output diminished, except during the fluctuations in lymph-flow above noted.

The results from the three animals form nearly a unit and are best discussed together. In all were observed:

1. A gradual decrease in the amount of lymph voided. This is no new finding.

2. A cell-concentration that varied little during the first $2\frac{1}{2}$ hours of lymph-fistula. Quantitatively the variation accords with the degree of cell-concentration involved, being greatest in Experiment I, with its high cell-concentration (averaging 6,981 cells per cubic millimeter, from which there is a variation of 997 cells, or 17.6 per cent.) and least in Experiment II (in which the cell-concen-

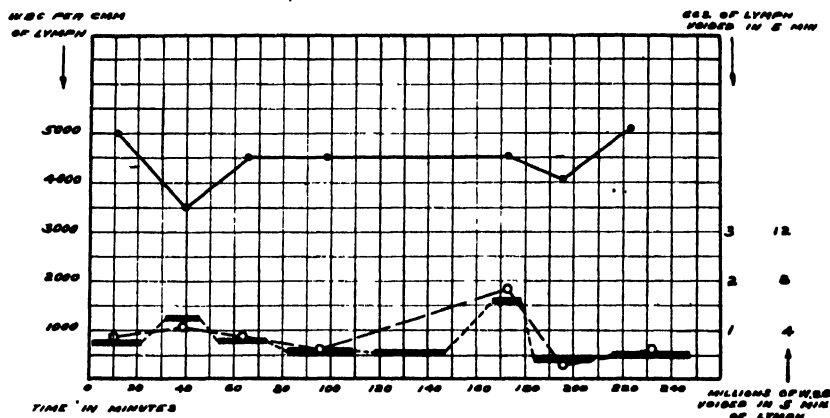


CHART 3.

tration averages 2,886 cells per cubic millimeter, and the variation is 506 cells, or 14.3 per cent.). In Experiment III the variation is 21.8 per cent.

So much for the cell-concentration during the first $2\frac{1}{2}$ hours. Later, in one case it rose markedly, in another fell slightly, in the third remained unvaried. The only previous counts under conditions somewhat similar are those of Forgeot already referred to. The animals he employed (ruminants) were not anesthetized, and his results show a variation, often of many thousand cells per cubic millimeter, in the counts from hour to hour. One understands this better on consideration of the small quantity of lymph he used in his estimations, the clotting that he frequently had to do with, and the absence of precaution to prevent struggle, which, as will later be shown, has a profound effect on the lymph's cell-concentration. Of fifteen animals on which he made observations, often over a period of more than 24 hours, the lymph in eight showed in general a tendency to lessened cell-concentration, in six, apart from large

transient variations, there was no change, and one exhibited an increase.

For our purposes it may be accepted that in the fasting dog anaesthetized with morphine and chloroform the cell-concentration of the lymph escaping from a fistula of the thoracic duct, is, during the first $2\frac{1}{2}$ hours, fairly constant, when the animal is quiet and the lymph formation is suffered to take place undisturbed. The variation in cell-concentration during this period is not greater than 25 per cent.

3. The total cell-output, apart from transient fluctuations dependent on those in the lymph-output and cell-concentration, showed a decided tendency to lessen.

Here an interesting point presents itself for discussion: "How is a constant cell-concentration maintained during $2\frac{1}{2}$ hours under the conditions of a slowing stream of lymph, and a diminishing total cell-output? The explanation is not evident.

According to Ehrlich, the lymph-cells, on their maturation, are caught up by the lymph-stream and transported passively into the blood; or, as he puts it in another connection, "one is obliged to conclude that a lymphocytosis occurs, when, in response to an increased circulation of lymph in a greater or less extensive lymphatic region, more elements are mechanically forced from the lymph-glands."³ Can one suppose that here the gradually lessening cell-output occurs because the lymph, through slowing, is rendered unable to force from the glands, and transport, its usual quota of cells? Or do the lymph-cells sediment along the course of the vessels in which they travel? Goodall and Paton, on the basis of counts from the receptaculum chyli, hold that some sedimentation in this reservoir is normal. Or do the lymph-glands, under the circumstances of the experiment, fail progressively in the maturation of cells? Any one of these happenings might explain the case.

Struggle, as will later be shown, increased promptly and markedly the cell-concentration of the lymph, although previously, under conditions of quiet, it had been lessening gradually. Thus it is

³The observations for an "active lymphocytosis" (Almkvist (19), Wolff and v. Torday (20), Prosser (21)) do not affect this conclusion, since they are concerned, not with lymphocytosis of the blood, but with the emigration of lymphocytes into the tissues and body-cavities.

shown that the glands are not lacking in cells fit for output. So the third hypothesis falls to the ground. One is left to explain in mechanical ways the constant cell-concentration in a lymph diminishing progressively in amount voided. One may suppose that the slowed current is not capable of transporting all of the many cells ready for it, and that, of those it picks up, some "sediment" on the way to the thoracic duct. The late rise in cell-content in Experiment II might, perhaps, be cited as an instance in which, despite these factors, the lymph became crowded with cells from the accumulation of those ready for it. In any event, the fact that the cell-concentration remains so long unchanged is surprising; one would expect to find immediately such variations as showed themselves only after several hours. Yet that the results are not (as might be supposed from Forgeot's work) examples of coincidence, is shown by the charts illustrating the effects of muscular exertion and of lymphagogue action. In these, despite varying cell-concentrations with varying physiological states, the same tendency to a constant cell-concentration is noted to occur hand in hand with a diminishing lymph-flow.

Discussion on the difference in average cell-output of the individuals will be reserved at this point.

With these results as a control, the effects of muscular exertion were taken up. It has been long known that this greatly accelerates the flow of lymph (Genersich, Lassar, Cohnheim (22)), but there have been no observations of its effect on the lymph's cell-content. A priori, on the theory that an increased output of lymphocytes is due, apart from special activity of the cell-forming tissues, to the flushing action of increased lymph-flow, one should find a transient increase in cells, traceable partly to those elements washed from the lymph-glands, and partly to those caught up from the channels by the swift current. But the existence of this increase, its amount, its duration, its effect on the blood, are all matters of conjecture.

In the experiments that follow, the animals were treated as those previously, except that they were at intervals made to struggle. Since morphia sufficed for the most part as anæsthetic, this was easily accomplished by giving a strong whiff of chloroform, or by tweaking the skin, or by an abrupt noise.

Experiment IV.—Irish setter; male; wt. 20 kilo; no food for 24 hours prior to operation. The thoracic duct was ligated, and a cannula introduced into it 5 minutes before the collection of lymph-specimens began. The lymph was slightly opalescent at first, later it was clear and yellowish; no clotting in cannula or tubes. The periods of struggle are noted on the chart. The cell-counts were made in the order in which the tubes were obtained, and between three and four hours after the collection of each one.

No autopsy done.

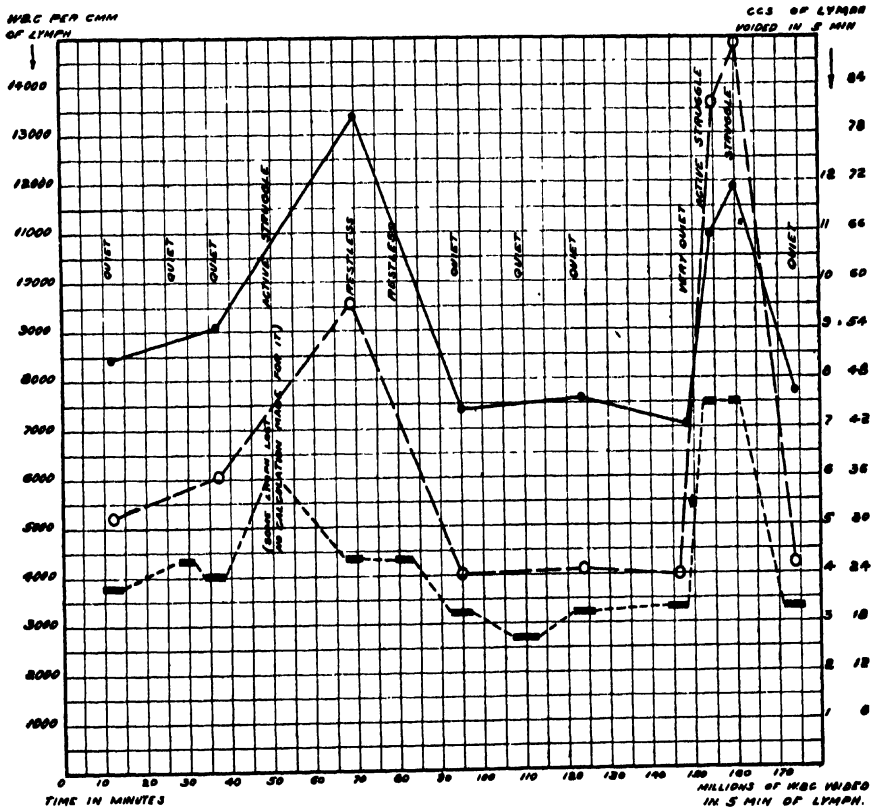


CHART 4.

In this instance struggle was twice induced. Each time the lymph-flow quickened abruptly and considerably, but with the return of quiet sank to below its former level. Each time, too, the cell-concentration became much greater. Thus the cell-output as a whole was multiplied. Indeed, it was necessary in this, and in the succeeding charts, to flatten the curve representing the cell-output.

with the beginning of struggle, nor with the greatest lymph-flow, but came later. Indeed, that induced by the second struggle appeared after exertion had ceased. This second struggle did not bring out such a flow of lymph or mass of cells as did the first (which was similar in intensity); and, following both, the lymph-output, cell-concentration, and cell-output, all sank to below their level previous to the exertion.

In the next experiment the struggle was purposely made very long.

Experiment VI.—Spaniel; male; wt. 11 kilo; no food for 24 hours before operation. The duct was opened 10 minutes previous to the experiment proper, after it had undergone 5 minutes ligation. With whiffs of chloroform and tweakings of the skin a continuous struggle lasting 35 minutes was maintained. The lymph was slightly chyloform throughout; no clotting in tubes or cannula. Cell-counts were made in the order of tube-collection and between 2½ and 4 hours after this. (See Chart 6.)

At autopsy several tape-worms were found in the intestines.

This chart, while in general like the others, shows that the increase in cell-output during struggle is neither transient nor small. It endured so that at the end of the 35 minutes exertion there were being emptied from the thoracic duct 1½ times as many cells in each 5 minutes as during the preceding quiet. In the 35 minutes of muscular activity 48 cubic centimeters of lymph, containing an average of 5,100 white cells per cubic millimeter, were voided, as compared with 21 cubic centimeters of lymph, containing 3,100 white cells per cubic millimeter, in the 35 minutes just previous, or slightly over twice as much lymph, and, in sum, nearly four times as many cells as when the animal was quiet. Immediately following struggle there was a great fall in the total cell-output, and during the next 50 minutes it held to a low level.

In this series of observations the effects of five struggles were noted, and, during the work on lymphagogue action (*q. v.*), the effects of three more. They agree in these results:

(a) Struggle causes the cell-concentration of the lymph to become much greater. An attempt was made to test the parallelism of this increase with that observed in the lymph-flow, by collecting specimens at short intervals of time. This was done in Experiments V, VI, and during the struggle in Experiment VIII. The

charts of these prove that the maximum cell-concentration appears after considerable struggle-lymph has been voided, and at a time when the rapidity of lymph-flow is lessening. In one instance it was present in the slowly flowing lymph obtained on the return of quiet. These facts bear on the problem of whether the

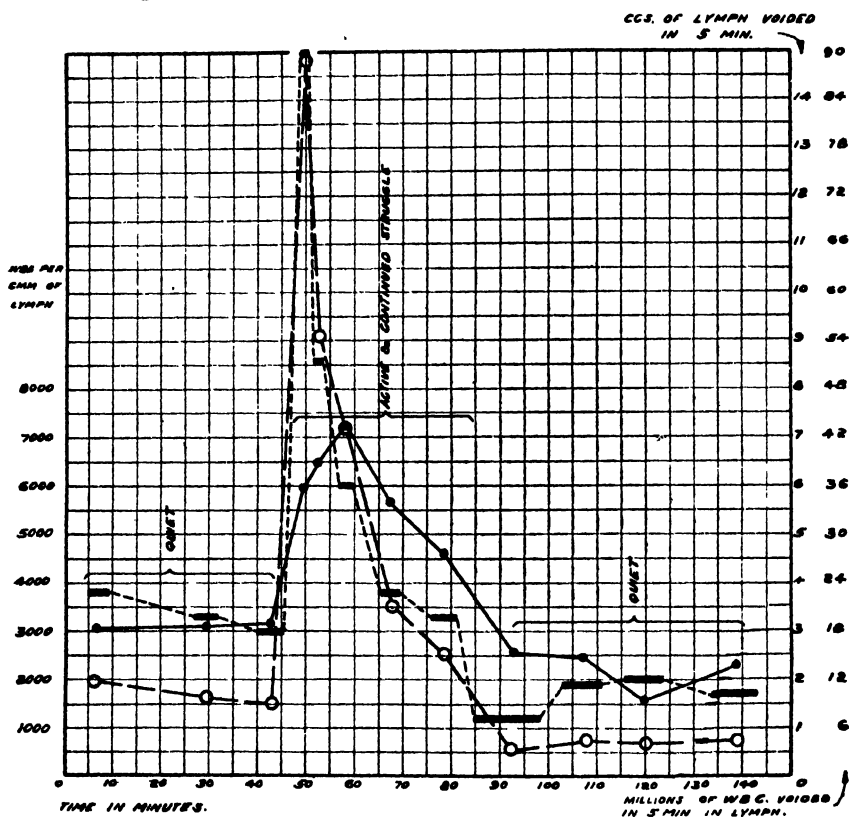


CHART 6.

cell-increase is due wholly to the flushing out of cells from the receptaculum chyli. Since the receptaculum is close to the opening of the thoracic duct, the cells flushed from it would appear, owing to the small size of the reservoir,⁴ in the first few cubic centimeters

⁴I have made notes on the size of the receptaculum in several freshly killed dogs. The size varies widely, as does the shape of the reservoir, which indeed may not be present as such (Jussifow (25), and others). The walls of the receptaculum normally are held nearly apposed, so that the content is slight; but an impediment in the thoracic duct causes almost immediate dilatation with the accumulation of 1 to 4 c.c. of fluid.

voided during struggle; and, following this evacuation, the cell-increase would disappear quickly. But, in reality, the maximum cell-output was not infrequently delayed in its arrival (Experiment IV, second struggle; Experiment V, first struggle), and the maximum cell-concentration practically always was. In addition, the cell-increase is not transient (see Experiment VI) as would be the case were it traceable wholly to elements sedimented in the receptaculum.

Even granted that the first addition in cells comes from the receptaculum, whence is derived the later addition? Recent evidence (MacCallum (23), Buxton and Torrey (24)) speaks against v. Recklinghausen's view that the peritoneal cavity opens by direct channels into the lymphatics; and the quantity of free cells of this cavity is, normally, very small. The bulk of the increase is doubtless derived from the further lymphatic ramifications, in particular from the lymph-glands and other centers of lymph-cell formation. That the increased cell-concentration may persist for a short while after quiet has been restored is not surprising, since a host of cells, started from peripheral regions on the journey to the thoracic duct, need not arrive there until several minutes after the cause that got them under way had ceased to act.

(b) Struggle causes the cell-output by way of the lymph to become much greater. This effect persists throughout a struggle of considerable length.

In explanation of this phenomenon of increased cell-output it must be remembered that the lymph-region drained by the thoracic duct during struggle is larger than that during quiet. During the latter state, according to Starling (26), the fluid that arrives in the duct is derived, practically in toto, from the abdominal viscera. But muscular movement immediately forces from the limbs much lymph (Lassar, Cohnheim, Winternitz), as well as from the viscera; and, following this effect of direct pressure, there is a secondary increase in the flow from both sources, due to new lymph-production (Heidenhain, Starling). The territory of cell-supply opened for the first time, so to speak, by struggle, helps account for the greatly swollen cell-output.

(c) Following struggle, the cell-concentration and cell-output become for a time less than they would have been in the absence of muscular exertion.

This is demonstrated in the charts of Experiments IV, V and VI. The lessening in cell-output might be deemed merely such as was seen in the control animals of the first three experiments, were it not that the cell-concentration and the lymph-output (of which two the cell-output is the product) are both lower for a time than they would have been in the maintenance of quiet. One may suppose the glands to have been deprived of the majority of immediately available cells, and the slowed lymph-stream insufficient to wash with it all of those actually present.

These findings are, further on, dealt with in their clinical bearing.

Experiments in which the rapidity of the lymph's flow is varied without movement by the animal should provide increased light on the mechanism responsible for the results just described. The lymphagogue action of glucose was accordingly turned to this purpose.

The period of observation in these experiments was so short that it could not matter if the glucose acted to stimulate or retard cell-development. Other factors, though, demanded consideration. A possible effect of a lymph of high sugar-content to loosen elements from the glands, through changes in osmotic relations, could not be ruled out. Further, the lymph of struggle and of "glucosæmia" are not derived in similar proportions from the same regions.

There are three great areas of lymph-supply (Starling). The liver; the other abdominal viscera, in special the intestines, whence the lymph of the whole region may be designated "intestinal"; and the remaining portions of the body, the lymph from which may be termed "extremity-lymph." As has been said, the lymph of struggle comes from all of these sources, and in no small part from the limbs. The intravenous injection of glucose gives also increased lymph-flow from all the sources (Starling). The results of the procedures might, then, be directly compared, were the tissue forming lymph-cells equally distributed. But the liver of the dog possesses none of this tissue except that in the glands at its hilus (Ellenberger (27)), whereas the intestines and mesentery are

quite rich in it. The other body-parts possess, in proportion to their bulk, a very moderate quantity. Thus, of "mixed lymphs" from the thoracic duct, those derived most largely from the liver should be poorest in cells. So one must ask whether the lymphs of struggle and of "glucosæmia" are exactly similar in their derivation. To this only an approximate answer can be given. Starling has found that of the lymph obtained after the injection of glucose, much comes from the liver, less from the intestines, and relatively little from the other portions of the body. According to him increased blood-pressure and differences in permeability of the capillaries are responsible for the whole phenomenon. On the other hand the abrupt, initial increase in lymph-output induced by struggle is largely dependent on lymph previously present in the limbs, and now forced from them by the movements. Nothing analogous to this is caused by the glucose. The persistence of the large lymph-flow during struggle is traceable, however, to the same cause⁵ as that following glucose injection, viz., increase in blood-pressure; and the resultant lymph is derived in much the same relative proportion from the three regions of production. Thus a rational basis is given to a comparison of the effects of glucose on cell-content with those observed in struggle after the initial increase in lymph-flow has subsided.

Experiment VII.—Mongrel; female; wt. 11.3 kilo; no food for 48 hours previous to experiment. The duct was ligated, and opened, 6 minutes before the beginning of lymph-collection. Lymph tinged with yellow, clear; no clotting in tubes or cannula. After three specimens had been got, 45 grammes of glucose in 72 c.c. of distilled water were injected slowly into the left subclavian vein. The animal remained absolutely quiet. The cell-counts were made in the order in which the specimens were taken, and 2½ to 3½ hours after their collection. (See Chart 7.)

At autopsy the animal proved to have been healthy; some round-worms were found in the intestines.

The results on cell-content are identical, as the chart shows, with those of struggle.

Experiment VIII.—Mongrel; female; wt. 16 kilo; no food for 48 hours previous to experiment. The duct was ligated during 10 minutes, and some stasis thus induced. On account of this, it was deemed safest to let the lymph

⁵ This assertion might justly be objected to by those who oppose the theory of the mechanical formation of lymph. It stands or falls with that theory.

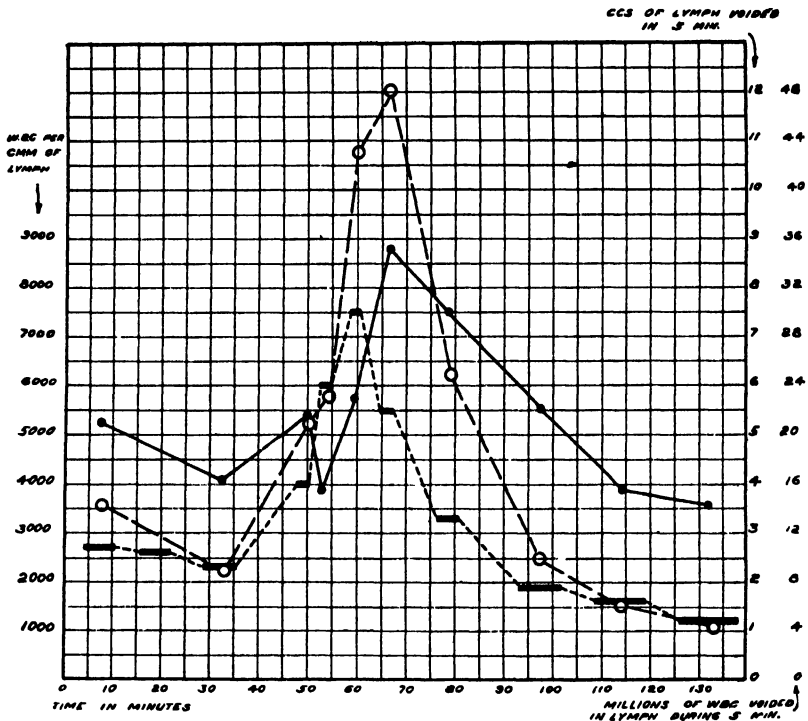


CHART 7.

escape for some minutes (12) before beginning its collection. Lymph clear; yellow-tinged; no clotting in tubes or cannula. After one specimen had been obtained, 52 grammes of glucose in 80 c.c. distilled water were slowly injected into the left subclavian vein. When the increase in lymph-flow due to this had subsided, the animal was made to struggle. Specimens were taken at frequent intervals. No chloroform was necessary, following its preliminary use to make anæsthesia complete. Cell-counts were made from the tubes in their order of collection and 2½ to 4 hours after that. (See Chart 8.)

At autopsy the animal was found to have been pregnant. There were ten embryos of an average length of 1 centimeter.

In this instance the effect of the glucose differed from that in Experiment VII. Here the cell-concentration rose, as result of the increased lymph-flow, whereas there it fell. In both cases the total cell-output became larger. In Experiment VII the curves were in all ways typical of those of struggle, whereas in Experiment VIII they were quite different, a fact which struggle in the same animal

helped to bring out. This struggle, coming after the glucose had operated, and causing an increase in the lymph but little superior to that from the glucose, was attended nevertheless by a cell-output vastly greater, and by the usual high cell-concentration.

These dissimilar results of glucose were puzzling. But the condition of the animal in Experiment VII had not been quite the

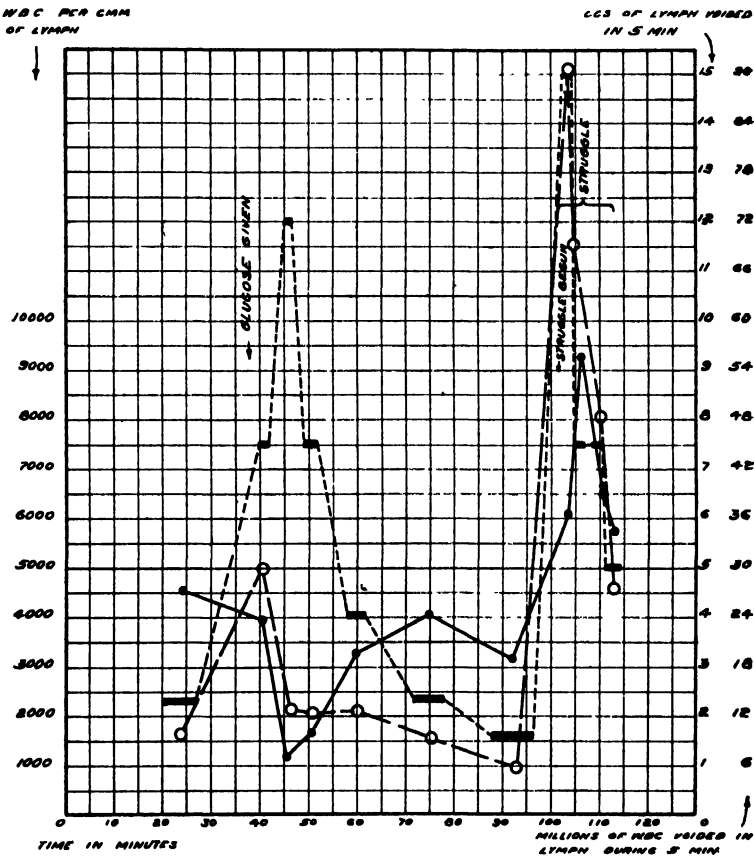


CHART 8.

same as that of the others. Owing to difficulty in the isolation of the thoracic duct, it had lain quiet under the anæsthetic 4 hours before the experiment proper began, instead of, like the others, only 1 to 2 hours. Perhaps, during this long period of preliminary quiet, the cells matured in the lymph-glands had in large part failed to be

carried away by the slow lymph-current, whence the marked appearance of them in the rush of fluid due to the glucose. On such reasoning it was determined to repeat the experiments, avoiding a long period of preliminary quiet, or flushing transiently the lymph-channels previous to the glucose injection by inducing restlessness in the animal.

Experiment IX.—Bull-dog; male; wt. 15 kilo; no food for 48 hours prior to experiment. The duct was ligated, and opened, 16 minutes before lymph-collection. The lymph was chyliform; no clotting in cannula or tubes. After one specimen had been obtained with the animal quiet, restlessness was brought

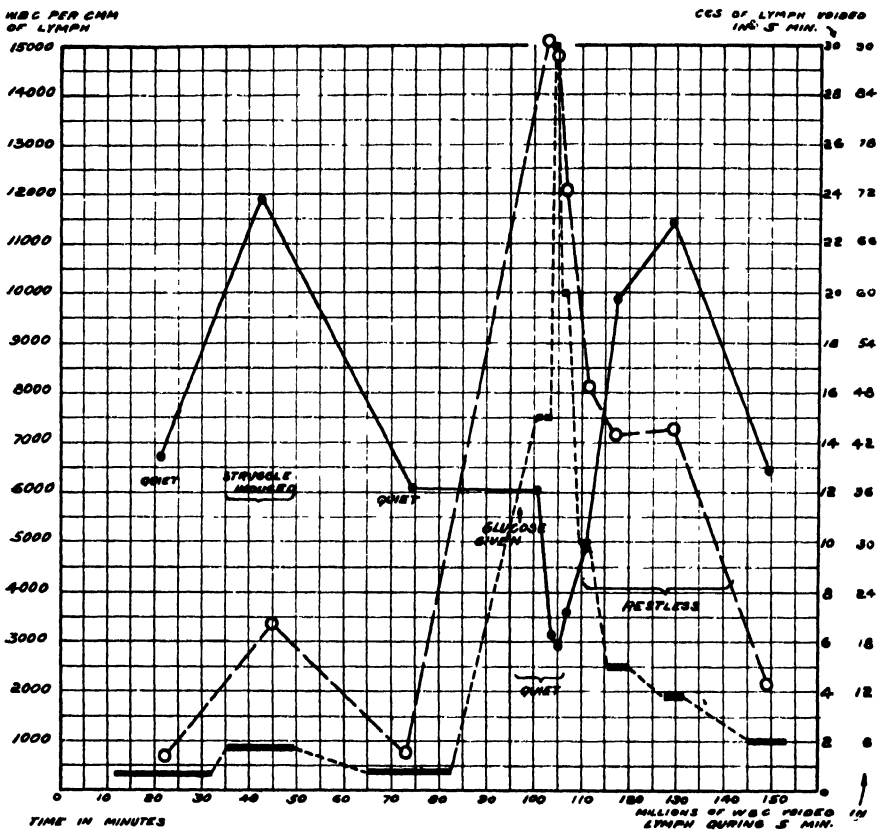


CHART 9.

about, in this case no real struggle, but a stiffening and straining, accompanied by labored respiration. The lymph became transiently more chyliform. A second specimen was now taken; on the return of quiet a third; and 50 minutes after restlessness had ceased 60 grammes glucose in 100 c.c. of distilled water were

injected slowly into the left external jugular vein. Before the lymphagogue action had disappeared the animal again became restless. Cell-counts were made in the order in which the specimens were taken, and $2\frac{1}{2}$ to 3 hours thereafter.

The dog at autopsy proved to have been sound. Several large pieces of bone in the stomach accounted for the chyloform lymph. A tape-worm was found in the intestine.

From the chart it will be seen that struggle brought its characteristic effects on the lymph's cell-content, despite an extremely small increase in rapidity of flow. With the enormous lymph-output caused by the glucose, the concentration of the separate cubic millimeter of fluid was diminished, yet the cell-output as a whole became profoundly more.

Experiment X.—Mongrel; male; wt. 24 kilo; no food for 24 hours before experiment. The duct was opened 18 minutes previous to the beginning of lymph-collection. It had been ligated 8 minutes. After observations during quiet the animal was made to struggle during $2\frac{1}{2}$ minutes, and 30 minutes later 100 grammes glucose in 100 c.c. of distilled water were injected into the left sub-subclavian vein. The lymph, which had been slightly opalescent, now became distinctly milky. There was no clotting in tubes or cannula. Except for the one struggle, the dog was quiet throughout. Counts of the tubes were made in the order of their collection and $1\frac{1}{2}$ to 3 hours following that. (See Chart 10.)

At autopsy one tape-worm was found in the intestines.

No count was made of the lymph taken during struggle. The curves representing glucose action are similar to those of Experiments VIII and IX. In this instance such a rush of lymph was observed, and such a vast increase in total cell-output (despite lessened cell-concentration), as showed itself in no previous experiment. The curves representing these are much flattened.

The results justify the supposition that the high cell-concentration seen in Experiment VII is traceable to accumulation of cells during the long, preliminary quiet. Experiment VII shows that under certain conditions increased lymph-flow^{*} may give results exactly similar, to those of struggle.

To summarize the results with glucose:

(a) The increase in lymph-flow produced by the intravenous injection of a solution of glucose is accompanied by an alteration in the cell-concentration of the lymph. Usually the cell-concentration

* The change in osmotic relations caused by the glucose cannot be ruled out as a possible factor.

is decreased, but there is some evidence to show that, when the conditions have been such as to lead to the accumulation of cells in the lymph-system, it may be increased.

(b) With the increased output of lymph there goes an increase in total cell-output. This increase may be enormous. In Experiment X $5\frac{1}{2}$ times as much lymph and $3\frac{1}{2}$ times as many cells, were

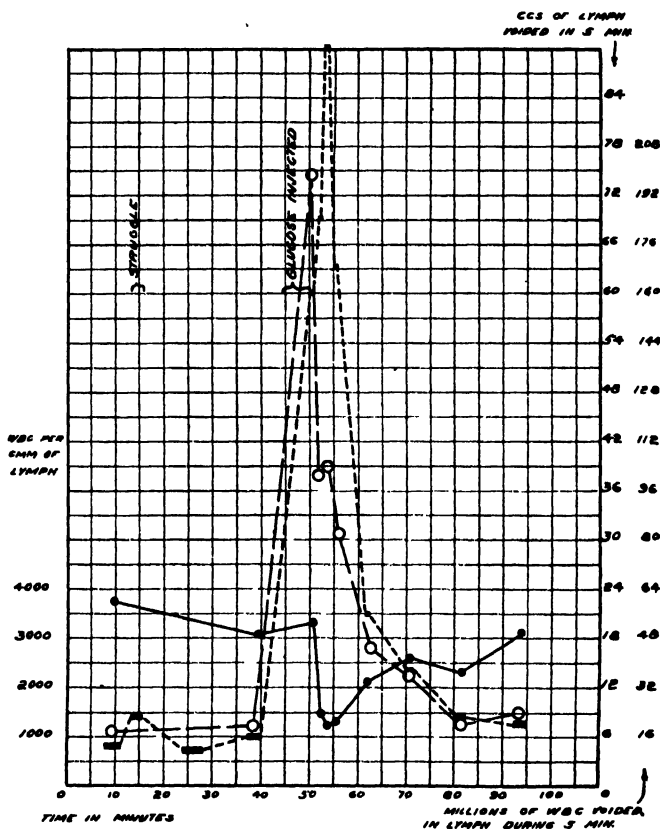


CHART 10.

voided in the half-hour immediately following the glucose administration as in the previous half-hour of quiet.

One may ask whether the results are not produced in the same way as those of struggle, and if they are not similar to them, except that the cell-concentration is rendered small by the distribution of the cells through a large amount of lymph. To put the question

directly, are the results of struggle those of simple increase in lymph-flow?

The evidence at hand is against this view. One may obtain a greatly increased cell-concentration during muscular activity in which the lymph-flow is quickened but little. Furthermore, increased lymph-flow by itself often diminishes the cell-concentration of the lymph, whereas struggle invariably heightens it. A direct comparison of the two procedures, such as Experiment VIII fortunately gave, demonstrates a difference in their results. In this experiment during the 11 minutes of muscular activity 19.25 cubic centimeters of lymph, with an average cell-content of 6877 cells per cubic millimeter, were voided; whereas, in the 11 minutes of greatest lymphagogue action, the nearly identical quantity of lymph voided (19.8 cubic centimeters) held only 2,267 cells per cubic millimeter. Differences in amount of lymph-flow alone could not be responsible for these variations of the cell-concentration in opposite directions and the differences in total cell-output.

It is true that, as already discussed, the lymphs obtained by the two methods may not be derived entirely from the same regions. The first "struggle lymph" is made up largely of that already present in the extremities, whereas that of the glucose is "liver-," "intestine-," and "extremity-lymph," in much the same relative proportions as that produced late in struggle.

It is questionable whether the cell-content of the "extremity-lymph" forced into circulation at the beginning of struggle could increase the cell-concentration of the "mixed lymph" of the thoracic duct, since it comes from a territory poor in lymphadenoid tissue, and has, indeed, been shown to hold normally fewer cells per cubic millimeter than does this "mixed lymph" (Pohl, Winternitz). But leaving this to one side, we are permitted a comparison of the effects of glucose and those that appear in struggle after the first rush of lymph has ceased. In the one instance the cell-concentration is diminished, in the other it is heightened.

One must conclude that another factor besides increase in lymph-flow per se helps during muscular activity to increase the cell-output. This is not hard to imagine. Direct pressure effects on the lymph-stream to set in motion those cells that had settled in the vessels,

and to scour the glands of mature elements, should certainly play a part. The work of Harvey (29) on the lymphocytosis caused by pilocarpine indicates that it is brought about by contraction of the smooth muscle in the capsules of the lymph-glands and spleen. Thus another possible action of struggle is suggested. Whatever the factor or factors may be, they are probably quite as influential during struggle to produce the large cell-output as is the accompanying increase in lymph-flow, which Ehrlich holds to be alone responsible for all heightened output of lymph-cells not dependent on their more abundant maturation. Nevertheless the theory that increase in lymph-flow gives increase in cell-output, is supported by the results of the lymphagogue action of glucose. The only objection to these as direct proofs of it is that an effect from the changes in osmotic relations brought about by a lymph of high glucose content cannot be ruled out.

It should be noted, in observing the charts as a whole, that the variations in cell-output are in keeping, quantitatively, with the amount of cell-output during quiet, the "cell-capital," so to speak. Succeeding to variations the cell-output tends to return close to its height previous to them. The fact that it usually becomes somewhat less than before them,—an indication of that gradual diminution in it observed in animals from which the lymph is gradually drained (Experiments I, II and III), or, in some cases (Experiments V and VI) of partial exhaustion of the supply of mature cells,—does not affect the principle. We may say that the cell-output seems "set" to maintain a stable rate during some hours at least. Healthy, adult dogs, kept, so far as possible, under the same conditions, differ widely in this rate of cell-output: in Dog G1 more than nine times as many lymph-cells per kilo of body-weight are furnished to the blood through the thoracic duct as in Dog F1. Does this mean a difference in amount of cell-production by the tissues? The appended table helps answer this question.

From this table it is clear that, while the cell-output per kilo of body-weight does not depend on size of the individual, or on differences in length of fast,⁷ it has perhaps a relation to rate of lymph-

⁷ Firleiwitsch (30) has found the lymph-glands of well-fed rats to be more numerous and larger than those of starved ones; but he assigns this to a larger size of the cells making up the tissue, not to a greater number of them.

Dog.	Length of Fast.	Weight in Kilos.	Average No. of w. b. c. per cmm. Lymph.	Average Flow of Lymph in 5 Minutes.	Total w. b. c. in this Amount of Lymph.	Total w. b. c. in Amount of Lymph Furnished per K. of Body- weight in 5 Minutes.	Flow of Lymph per K. of Body-weight, in 5 Minutes.
Ll, bull, male	48	15	6,700	0.7	4,690,000	312,666	0.05
Hl, terrier, male	24	11	4,960	0.75	3,720,000	338,181	0.07
Pl, collie, male	48	13	2,900	1.5	4,350,000	334,615	0.11
Al, bull, female	24	15	3,600	2.0	7,200,000	480,000	0.13
Kl, collie, male	48	16	4,510	2.3	10,373,000	648,313	0.14
Gl, bull, male	24	18	11,160	3.3	36,828,000	2,046,000	0.18
El, setter, male	24	20	8,400	3.75	31,500,000	1,575,000	0.19
Ml, setter, male	24	24	3,780	4.6	17,388,000	724,500	0.19
Ol, collie, male	48	23	6,760	4.6	31,096,000	1,352,000	0.20
Cl, mongrel, male	2	10	1,500	2.0	3,000,000	300,000	0.20
Nl, terrier, female	48	8	4,180	1.7	7,106,000	888,250	0.21
Jl, mongrel, male	48	11.3	4,640	2.5	11,600,000	1,026,540	0.22
Fl, mongrel, male	24	19	990	4.3	4,257,000	224,053	0.23
Il, spaniel, male	24	11	3,000	3.8	11,400,000	1,036,363	0.34

flow per kilo of body-weight, being, on the whole, least in those individuals in which this flow of lymph is least. It may be supposed, in the light of what has gone before, that the lymph-flow, as an agent of transportation, is here responsible for the differences in cell-output, rather than by stimulation of the cell-forming tissues; in special since, so far as we know, this rate of cell-output is constant only from hour to hour, and may not be so from day to day. Some of the wide differences may be due to the presence of an accessory thoracic duct, which is not infrequent in the dog (Biedl and v. Decastello), or to other channels conveying a share of the elements that would, normally, course through the thoracic duct. In association with the idea of actual variation in the productive activity of the cell-forming tissues, may be cited the work on ruminants of Forgeot, already quoted. He found the cell-output of young individuals to be markedly greater than that of adults. Differences in age of the animals may be at the root of some of the differences in cell-output. But the mechanical factors, just cited, will explain the larger differences, and make needless a further entrance into the dark subject of activity in the cell-forming tissues.

The cell-concentration of the lymph of the dog, as determined under the conditions of operation outlined at the beginning of this paper, has a worth in its relation to the cell-content of the blood.

Delamere, Winternitz, Goodall and Paton, Dastre, Henri and Stodel, Beidl and v. Decastello, and Ranvier give figures ranging from 1,372 to 22,729 white cells per cubic millimeter. The results in the table, as might have been expected from the technic employed, do not exhibit such wide variation. From them one may judge this "normal" cell-concentration of the "mixed lymph" of the dog to lie between 990 and 11,160 cells per cubic millimeter, with an average of 4,000 cells.

The most important outcome of this work is the discovery that the system forming the lymphocyte possesses large reserve power to increase transiently its output of cells. During muscular activity this may, for the space of a half-hour, be nearly four times what it is in quiet, as has been shown. A clinical application of the finding is not far to seek.

For this application it is necessary to know what white cells the lymph furnishes the blood. These are in the dog much the same as in man, just as the leukocytes of the dog's blood resemble in general those of man both in morphology and in relative proportion of number (Dawson (31), Tallquist and Willebrand (32), Busch and Van Bergen (33)). The bulk of cells furnished through the thoracic duct of the dog is one of lymphocytes, large and small, but a few large mononuclears, a varying, small percentage of eosinophiles, and an occasional polymorphonuclear neutrophile are also present (Delamere, Biedl and v. Decastello). The lymphocytes alone exist in sufficient quantity to be important to the blood.

Since, as has been emphasized, the thoracic duct furnishes a large, if not the greater part, of the blood-lymphocytes, an increase in the lymph's cell-concentration should produce, other factors being equal, an absolute lymphocytosis in the blood. When the amount of lymph is at the same time increased, thus multiplying the cell-output, the effect should be more profound. Thus one would expect struggle to produce, clinically, an absolute lymphocytosis.⁸

⁸ But it must be assumed that the change in cell-output is not accompanied by change in cell-formula. I have made repeated counts of the lymph before and during struggle, and have found no such change.

The clinical records of blood-counts following muscular exertion indeed show the presence of an absolute lymphocytosis. Schultz (34), and later Winternitz, observed a leukocytosis following exertion, but they did not note its kind. Burrows (35) found in the normal individual, after short exercise, a distinct increase in number of all the white cells, but especially of the lymphocytes. Capps (36), previously, had studied the blood during the convulsions and apoplectic attacks of general paresis, and, here too, had observed leukocytosis, most marked in the large mononuclear elements, but affecting the lymphocytes. Burrows went further in making plain the fact that the leukocytosis associated with true convulsions in the course of paresis is invariably of the inflammatory type, the polymorphonuclear neutrophiles giving the increase. He concluded, from his findings made during muscular exertion, that in convulsions two leukocytoses are really involved, a transient "physiological," wherein occurs an increase of all the elements, most marked, as his records show, in the lymphocytes, and a more pronounced and enduring "pathological" one.

Violent and long-continued physical exertion will itself produce a profound leukocytosis. Thus Larrabee (37) observed it as an effect of a 25-mile foot-race; and he decided that, here too, a "pathological" is superimposed upon a "physiological" leukocytosis, "an increase in cells all along the line," as he puts it. Only this "physiological" leukocytosis is of interest here. Larrabee, Tileston and Emerson (38), studying the blood after a similar race, confirmed these results. Further, the lymphocytes at the end of such exertion form a very small percentage of the total, having retreated to their absolute number during quiet, or even below it. We do not know how far destruction of the lymphocytes in circulation effects this and how far it is brought about by a lessening in the supply of them. The experiments reported in this paper show that a diminution in the cell-output by way of the lymph follows prolonged struggle.

Coming to more debatable ground, the well-defined lymphocytosis that accompanies whooping-cough may be referred to. This cannot be primarily due to the mechanical effects of struggle, since it appears early in the disease; yet the fact that it is at its greatest during the period of violent coughing (Meunier (39)), is suggestive. Un-

fortunately there are no published figures dealing with the leukocyte count immediately before and after a coughing-fit.

The experiments with a lymphagogue throw a little light on the vexed subject of digestive leukocytosis, particularly on the reason that it is vexed. The frequency of this leukocytosis, and the part played in it by the mononuclear element, varies with nearly every investigator who has applied himself to the problem. The discordance in results is, to a certain extent, explicable in terms of the conditions dealt with here. Quiet previous to a meal would predispose, on increase of the lymph flow during digestion, to a cell-output such as that in Experiment VII, which would probably swell quite markedly the number of lymphocytes in the blood. Any exertion after the meal would tend to make this output greater. Exercise previous to a meal, by flushing out the reserve of mature cells, would act to prevent an increase in cell-output during digestion, and this result would be made the surer by slow lymph-flow, were but little fluid ingested with the meal. Under such circumstances the blood-content in lymphocytes would not increase.

I am aware that, considering the many factors which must enter into the determination of the blood's content in lymphocytes, this discussion is one-sided. Yet, whether the hypotheses presented above be sound or not, the work on which they are based indicates one direction in which it may be possible to simplify some of the problems connected with the leukocyte.

CONCLUSIONS.

1. The lymph of the thoracic duct furnishes to the blood a larger proportion than is usually supposed of the lymphocytes in circulation. Gross variations in its output of such cells must affect very considerably the blood picture.

2. The quantity of lymphocytes supplied through the thoracic duct of the healthy dog remains practically constant from hour to hour, if the physiological conditions are not notably changed. Transient change in physiological conditions may alter the output of cells, but with the disappearance of this change the output tends to resume its previous rate. These facts indicate that the tissues producing lymphocytes are "set" at a rate of activity definite in the individual.

3. Muscular activity (struggle) produces a prompt increase in the output of lymphocytes through the thoracic duct.

(a) This is assured by the presence of an increased number of cells per cubic millimeter of lymph, combined with an increase in the amount of lymph voided.

(b) The lymphocyte-output may be tripled or quadrupled during a long-continued struggle.

(c) Following prolonged struggle the output of lymphocytes is for a short time less than previous to the exertion.

4. The increased lymph-flow caused by a lymphagogue of the second class (glucose) brings with it increased output of lymphocytes through the thoracic duct.

(a) The individual cubic millimeters of lymph are often poor in cells, during the rapid lymph-flow, yet the total number of elements transported is large.

(b) The results with glucose support the theory of Ehrlich, that a rapidly appearing lymphocytosis may be produced through the flushing effect of increased lymph-flow.

5. A comparison of the effects of struggle with those of glucose demonstrates that in the former some factor besides increase in lymph-flow per se (Ehrlich) works to cause the large output of lymphocytes. The nature of this factor has not yet been determined.

6. The variations caused by muscular exertion and by increased lymph-flow in the number of lymphocytes coursing through the thoracic duct are so pronounced as to suggest that the total number of lymphocytes in circulation must be considerably influenced by them. Clinical findings by other observers indicate that this is true; and the clinical findings themselves become much simpler of interpretation.

7. The results in general prove the existence, reserved from circulation, of a large fund of lymphocytes, which is quickly yielded to the blood under certain physiological conditions.

I wish to thank Dr. Warthin for an interest in the work that has been most helpful.

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CALCIFICATION OF THE ARTERIAL SYSTEM IN A
CAT WITH TRANSPLANTED KIDNEYS.

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CALCIFICATION OF THE ARTERIAL SYSTEM IN A CAT WITH TRANSPLANTED KIDNEYS.¹

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In a previous paper,² the history of a cat,³ whose kidneys were extirpated and replaced by the kidneys from another cat, and whose arteries underwent afterwards extensive calcification was incompletely reported. It was the first observation showing that a very slight morphological change of the kidney might be followed by intense and rapid arterial degeneration. On account of the importance of this fact it seemed advisable to summarize the clinical history of the animal and to describe fully the gross and microscopical findings of the autopsy.

The animal operated on was a young adult female cat in excellent health which had lived in the laboratory for several months. Her kidneys were resected, the abdominal aorta was dissected and found normal, and both kidneys from a middle aged female cat, which was also in good health and whose arteries were normal, were grafted into her abdominal cavity. The animal recovered quickly from the operation and her life went on just as before. Fifteen days after the operation, both kidneys were movable and normal in size. The animal urinated and lived as a normal cat. Seventeen days after the operation, both kidneys were found very much enlarged and fixed to the lumbar wall, and the urine contained a great deal of albumin. An exploratory laparotomy, performed on the eighteenth day, showed both kidneys very much increased in size. Their consistency was softer than normal. The lumbar peritoneum was incised on the middle line, and dissected. The arterial and venous circulations appeared to be normal. The connective tissue of the hilus was œdematous, and clear fluid flowed

¹ Received for publication, January 7, 1908.

² Alexis Carrel, *Jour. of Exper. Med.*, 1908, x, 98.

³ Experiment 14.

from it. The wall of the ureter was œdematous also. The color of both kidneys was rosy and normal; there was no congestion. The peritoneum covering the anterior face of the right kidney was dissected and retracted and the capsule incised; clear fluid and red blood flowed from the incision. Then the renal tissue was incised and found œdematous, but not congested. An abundant hemorrhage of red blood followed, which was controlled by suture of the capsule with very fine silk. No suture of the lumbar peritoneum was made. The abdominal wound was closed as usual. After the operation, the quantity of albumin decreased rapidly. The size of the kidneys diminished progressively and was almost normal fifteen days afterwards. Nevertheless the animal became emaciated, and, without having presented any definite symptoms, died on the thirty sixth day after the transplantation.

The autopsy was performed one hour and a quarter after death.

Macroscopical examination.—On the abdominal wall, two transverse scars are observed. The lower one is linear and hardly discernable; it is the scar of the first laparotomy. The upper one, a little wider and very apparent, is the scar of the second laparotomy. To palpation, these scars present extremely different characteristics. The scar of the first laparotomy is a normal, narrow and elastic scar, while the scar of the second laparotomy is very wide, irregular, and its consistency is extremely hard, as if a rib had developed in the abdominal wall.

After incision of the wall, it is found that the scar of the first laparotomy is entirely normal, without any apparent infiltration of lime salts. On the contrary, the scar of the second laparotomy is so hard that it is difficult to cut it with the scissors. The wall is infiltrated with masses of lime salts, white in color and almost as compact in structure as a stone of the urinary bladder. Nevertheless, at the level of this scar, the peritoneum appears to be normal. It presents a white color, which is due merely to the deposit of lime salts in the subperitoneal connective tissue.

The abdominal cavity was opened by a large transversal incision. There are a few adhesions of the great omentum to the scar of the second laparotomy and no adhesions of the intestine. There is no fluid in the abdominal cavity and the parietal peritoneum is normal everywhere.

The small intestine was eviscerated. Its connections are normal. However, a loop of the first part of the jejunum is adherent to the scar of the lumbar peritoneal incision made during the second operation, and is sharply kinked at the level of the lower part of this incision. Nevertheless, there is no obstruction of the lumen of the intestine. The large intestine is normal. The omentum and mesentery are normal. The superior mesenteric artery is larger than normal; its consistency is very hard, and it can be broken by pressure as though it were a glass tube. It seems also that the consistency and size of the smaller mesenteric branches are markedly increased.

The stomach is apparently normal, but its arteries are dilated and very hard. The liver, the spleen and the pancreas are normal.

The kidneys are in their normal position and covered with normal peritoneum. The cat being emaciated, there is little adipose tissue around the kidneys, and the peritoneum is very transparent. The color of the organs is normal, and also their consistency. Their size is slightly enlarged, but within normal limits. They are movable on the lumbar wall, as normal kidneys are. The loop of jejunum which was adherent to the incision of the second lumbar peritoneal incision having been detached, it is found that the scar of this incision is markedly infiltrated with lime salts. By examining more carefully the peritoneum covering the right kidney, a white longitudinal spot is seen about two centimeters external to the hilus. This spot is hard in consistency and composed of a deposit of lime salts in the subperitoneal connective tissue, between the peritoneum which is sound and the capsula of the kidney which also is normal. This deposit of lime salts corresponds approximately to the place where the vertical subperitoneal exploratory incision of the right kidney was made. The healing of this incision has been so perfect that very little evidence of it can be found by a most careful examination in the capsule or in the renal substance. Its location is marked only by the infiltration of lime salts into the subperitoneal tissue, which has followed probably a very small hemorrhage from the line of suture of the capsule. The renal veins and arteries are normal in size, direction and consistency. The connective tissue surrounding them is normal also. The transplanted segments of aorta and vena cava are normal, and the anastomoses are excellent. But, the line of suture of the arterial anastomoses is as hard as a ring of wire, and there is a sharp difference at this point between the transplanted aorta which is elastic and soft, and the host's aorta which is as hard as glass.

The ureters are normal. A few centimeters below the kidneys, they become adherent to each other and enter the peritoneal cavity through the lower part of the lumbar peritoneal incision. After having passed along the right side of the rectum and above the uterus, they reach the bladder. They are in excellent condition and their small vessels appear to be normal. The transplanted flap of bladder is perfectly united to the bladder which is normal in appearance and in size. Its anterior face is longitudinally incised. About five cubic centimeters of yellow clear urine, containing albumin are found. The openings of the ureters are distinctly seen and are normal. The transplanted mucous membrane is normal and the scar between it and the mucous membrane of the host is almost undiscernable.

Then the kidneys were dissected and directly examined. The capsule is normal in appearance. At the level of the anterior face of the left kidney, there is a narrow brownish line which is probably the scar of the exploratory incision. The adhesion of the capsule to the kidneys is normal, and not increased at the level of the scar. There is no dilatation of the stellate veins. Both kidneys were opened. There is no dilatation of the pelvis. The relative dimensions of the cortex and medulla are normal. The medulla is normal. The cortex is normal but a little pale.

The suprarenal glands are normal.

In opening the thoracic cavity it is found that the cartilaginous part of the ribs is very hard and friable and intensely calcified. The internal mammary arteries are also calcified. The pleura, the trachea and the lungs are apparently normal. On a macroscopic section, the pulmonary tissue seems normal, but, on palpation its consistency is found to be greatly modified by the presence of a great many very small and hard foreign bodies. These were proved to be the calcified ends of the small bronchioles. The larger bronchi are normal, and their rings do not present any calcification. However, thick calcific nodules are found from distance to distance on the external layer of the larger bronchioles.

The heart is very small, much smaller than the heart of a normal cat of similar size. There is no apparent calcification of the coronary arteries. The valves are normal. Nevertheless, as the aorta is very much dilated, there was probably a marked degree of aortic insufficiency. The arch of the aorta, the brachio-cephalic and carotid arteries, and the descending aorta, have the consistency of glass tubing and are exceedingly friable. The arch of the aorta was incised longitudinally. It is very hard to cut with the scissors, especially near the heart just above the sigmoid valves. There is at this level a very large accumulation of lime salt. The dimensions of the vessel are very much increased. The internal circumference of the aorta near the heart is 26 millimeters, the same dimension in a normal cat being about 18 millimeters. The internal surface is of a yellowish-white color, with a reticular appearance, the elevated parts being more yellow and harder, while the depressed parts are whiter and a little softer. It corresponds to the differences in the intensity of the lime infiltration. But the wall, over the entire circumference of the vessel, is infiltrated and rigid. It is not a lesion localized in a few patches, but a diffuse one generalized over the whole vessel. However, near the mouth of the brachio-cephalic arteries there are thicker rings of lime infiltration. On the descending aorta, the infiltration is a little less marked and more regular.

The wall of the aorta is thinner than normal in several places. On the upper part of the arch, which is very much dilated, and, just in front of the mouth of the left brachio-cephalic artery, there is a part so thin that it would have probably broken under the aortic pressure had the animal lived a little longer.

The lesions of the pulmonary artery are very different. The dilatation is not marked and the wall has an almost normal consistency. But there are intense focalized calcific lesions. Just above the sigmoid valves, on the convex part of the vessel, there is a long calcified patch of yellowish color, seven millimeters in length, and raised above the intima to a height of more than one millimeter. Other patches are observed near the bifurcation of the pulmonary artery and in its branches. Between the patches the wall seems normal. The smaller branches of the pulmonary arteries are found enlarged and infiltrated with small white patches.

Diffuse calcification and thick circular rings are found on the wall of the carotid arteries. The subclavian and humeral arteries assume the same appearance. Even the small muscular branches of the humeral arteries are calcified.

The abdominal aorta presents diffuse lesions almost similar to those of the carotid and subclavian arteries, that is, diffuse calcification with thicker rings from distance to distance. These lesions stop sharply at the point of the anastomoses, and the transplanted aortic segment is soft and normal.

The calibre of the abdominal aorta is very much increased. The cœliac artery is calcified and dilated. The upper mesenteric artery is also completely calcified, and its lumen is as wide as this of a normal aorta.

The iliac and femoral arteries are affected with similar lesions.

Microscopical Examination.—The specimens were fixed in Zenker's fluid and in formalin and stained in hæmatoxylin and eosin. The arteries were cut without having been decalcified. Some of the arterial sections were also stained with Weigert elastic tissue stain. Dr. Simon Flexner had the kindness to look over the sections for me.

The Kidneys.—Beneath the renal capsule, in the superficial part of the cortex, there is a marked œdema which extends about one half millimeter downwards and then disappears. It is not regularly diffused but is more marked in some places than in others. There is also a focalized increase in connective tissue immediately beneath the capsule, chiefly about greatly dilated blood vessels, with heavy fibrous walls. The latter are dilated superficial veins. Surrounding them, there is a rich infiltration of small round cells. In one of these small veins, an organized thrombus projects into the lumen of, but does not occlude the vessel.

The secretory tubules are on the whole remarkably well preserved and there is no increase in connective tissue around them. On the other hand, groups of collecting tubules, high in the cortex, occasionally show an increase in the surrounding connective tissue, dilatation and either casts or cast material, which are composed in part of disintegrated blood corpuscles. Following the collecting tubules into the medulla, one finds focalized infiltrations around them of small round cells.

In the glomeruli, Bowman's capsule is not increased in thickness. For the most part, the capillary loops fill the capsule. The endothelium stains sharply and the capillaries contain blood corpuscles and no excess of leucocytes. The capillary walls are not thickened. There is no fluid in the capsular space.

No calcification is detected in any of the renal structures.

The liver, the spleen, and the intestines are normal. There is no change in their small vessels.

There is no lesion of the lungs. But the cartilaginous rings and plates of the middle sized and small bronchioles are intensely calcified. There is no calcification of the rings of the large bronchi and of the trachea. A section of the upper part of the cervical medulla is found normal and there is no lesion of its small vessels.

The suprarenal glands are entirely normal.

The Arteries.—The segment of aorta which was examined belonged to the middle part of the descending thoracic aorta. The intima appears normal. The muscular coat shows a very great reduction in the number of the nuclei ordinarily present. This reduction begins to be visible in the layer beneath the endothelium and becomes progressively more marked as we proceed outwards toward the adventitia. As the nuclei disappear, the muscular cells become more and more confluent and converted into a highly refractive material. As the adventitia is approached, lime salts begin to be deposited. The external musculo-elastic layers of the media, and the adventitia are extensively infiltrated with lime salts. There is a less degree of calcification, consisting of the deposit of fine

lime granules, in the middle part and more internal part of the media. This latter is not uniform and does not entirely enclose the vessel. Where the lime deposit is greatest, there is an abundant infiltration of small cells, some of which show polymorphic nuclei. The cells are in a poor state of preservation, and these focalized areas correspond more or less closely to the so-called atheromatous abscesses. A section stained for elastic tissue shows that the coarser elastic fibers remain but are especially prone to rupture in the course of section. In the location described as resembling atheromatous abscess, the elastic tissue is deficient or very much broken up. The smaller blood vessels about the aorta do not show any calcification.

The carotid artery shows a condition somewhat different to that of the aorta. The entire muscular coat of the artery has lost its nuclei. Not one remains. It is converted into an homogeneous structureless membrane richly infiltrated with lime salts. The adventitia is relatively free of calcification. The elastic tissue stain shows that the elastic framework remains but the fibrils have coalesced together into stands of greater or less thickness, showing in transverse sections a parallel arrangement. There is no fine network present.

No section has been made at the level of the most marked lesions which took place on the arch of the aorta, and on the pulmonary artery.

No attempt will be made to explain the genesis of these degenerative arterial changes. The problem contains too many unknown quantities, and hypothesis, however ingenious, would have very little objective value. Nevertheless a few details of this observation must be emphasized, especially as regards the time of occurrence of the calcification, its localization, and its relation to the renal lesions.

During the first fifteen days following the double transplantation the animal was in normal condition and no calcification took place as was shown by the anatomic conditions at the second laparotomy and by the normal structure of the scar of the first laparotomy. Then, the kidneys became suddenly enlarged, and the lesion noted at the laparotomy performed on the eighteenth day was oedema of all the transplanted tissues, for which no mechanical cause was discovered.

The calcification took place during the eighteen days which elapsed between the second laparotomy and the death of the animal.

The calcification was localized to the arteries, costal and bronchial cartilages, and to the points where the tissues had been disturbed by incision, or even merely by infiltration of a little blood. It was a remarkable fact that no calcification was found in any of the transplanted tissues. The transplanted segment of aorta,

and the renal arteries were normal. The subperitoneal connective tissue of the host and the connective tissue of the hilus of the right kidney were dissected during the second operation. However the connective tissue of the hilus was found normal at the autopsy, while the subperitoneal tissue was infiltrated with lime salts.

The exploratory incision of the right kidney was perfectly cicatrized, and there was no calcification at all of the renal tissue or the capsule. But at the level of the incision in the subperitoneal connective tissue there was a patch of lime salts. The blood, which flowed from the edges of the renal structures, had produced a deposit of lime salts in the tissue of the host, while it was harmless for the transplanted tissue.

It must be noticed also that the pathological changes undergone by the renal tissue were slight. However, the kidneys are evidently responsible for the occurrence of the arterial degeneration, directly or indirectly. Therefore, this observation seems to show that some unknown condition of the kidneys corresponding merely to unimportant morphological changes, can produce rapid, grave and extensive arterial lesions associated with deposits of lime salts in them and in other locations.

THE EFFECT OF PILOCARPINE ON THE OUTPUT OF LYMPHOCYTES THROUGH THE THORACIC DUCT.*

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The lymphocytosis induced in the blood by pilocarpine is a phenomenon which has been turned to the use of many theories but has had, of itself, little study. Horbaczewski (1891) (1) discovered that the drug increases the white cells. The finding helped him to work out his idea of the dependence of uric acid excretion on leukocyte destruction. Ruzicka (2) obtained a profound leukocytosis in rabbits by the intravenous injection of large doses of pilocarpine. He could not account for the rapid occurrence of this result, but thought proliferation in the hæmatopoietic tissues responsible for its continuance. Waldstein (1893) (3), assuming that an increase of the mononuclear elements in the blood would influence favorably the course of some infectious diseases, gave small amounts of pilocarpine at intervals of several days, and obtained as result a large, absolute lymphocytosis. Recently Lefmann (4) and Gasis (5) have repeated Waldstein's experiments with rabbits, in demonstration of an effect of the Roentgen rays to bring about quick disappearance of the lymphocytosis.

The evidence seems good that pilocarpine given in small doses over a considerable period of time produces a lymphocytosis absolute in type. The immediate effect of the drug is to cause (in rabbits) a general increase of the white cells, involving especially the lymphocytes. This result is often cited as an example of possible chemiotactic influence on the lymphocyte, but Harvey (6) has produced evidence to prove it due to contraction of the smooth muscle of the lymph-glands and spleen. Unfortunately his work

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is based on counts of but one hundred cells each from blood smears. There have been no other investigations on the cause of the quick change in the blood picture.

A method adopted by the author (7) for study of the cell-output through the thoracic duct has given him opportunity to note in dogs the immediate effect of pilocarpine on this source of the lymphocytes. In brief, considerable quantities of lymph (3 cubic centimeters to each specimen) are collected from the thoracic duct in specially graduated tubes containing 3 cubic centimeters of 4 per cent. sodium citrate solution in 0.8 per cent. salt solution, and the white cells per cubic millimeter estimated from the mixture after it has been thoroughly agitated. This estimation is accomplished with melangeur and counting-chamber in the ordinary manner. The mixture of lymph and sodium citrate solution is not diluted, but the addition to it of a trace of a saturated aqueous solution of methyl violet (5B) facilitates the counting. The accuracy of this method of cell-enumeration, and the slight variation in the number of cells per cubic millimeter of lymph voided from the thoracic duct during the first two hours after the establishment of a lymph-fistula in an animal quiet under morphia and chloroform, have been shown in the paper cited. The output of cells as a whole becomes gradually less during this period, as the amount of lymph voided gradually lessens.

The dogs used were given, one hour before operation, 0.5 centigram of morphia sulphate per kilo of body weight, and later chloroform to complete the anaesthesia. A cannula was introduced directly into the thoracic duct; the lymph allowed to flow free; and, after the collection of one or more specimens, a small dose of pilocarpine nitrate, dissolved in a few minims of salt solution, was injected intravenously and further collection of lymph made. In every instance the action of the drug was evident within the minute through increase in the saliva.

Since figures dealing with the effect of pilocarpine on the blood of the dog are lacking, preliminary counts were obtained on animals treated as above outlined, except that no lymph-fistula was produced, and the only operation was that necessary to give access to the left, external jugular vein, into which the injections were made.

Throughout the term of observation the animals were kept quiet under morphia and chloroform. In each instance food was withheld during the twenty-four hours previous to operation. Blood for the counts was obtained by nicking with scissors small superficial veins on the abdomen. The cover-glass preparations were stained with Wright's stain, and for each differential count (of 500-600 cells) at least two smears were used. In the table that follows the number both of large and of small mononuclear cells is given.

Experiment I.—Bull-dog, female; wt. 16.5 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.	Rbc. per cmm.
A. M. 9:50	Operation.					
10:15	First count.	18,600	986	484	1,490	6,672,000
10:45	Second count.	18,200	746	528	1,274	
10:50	Pil. nit. 20 mg. intravenously.					
11:22	Third count.	24,900	1,619	896	2,515	6,960,000

Experiment II.—Fox-terrier, male; wt. 9 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.
A. M. 9:40	Operation.				
9:48	First count.	6,400	749	179	928
10:23	Second count.	9,720	846	136	982
10:26	Pil. nit. 10 mg. intravenously.				
11:13	Third count.	15,200	1,870	274	2,144

Experiment III.—Pointer, male; wt. 22 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.
A. M. 9:30	Operation.				
10:30	First count.	13,000	959	429	1,388
10:35	Pil. nit. 10 mg. intravenously.				
11:05	Second count.	19,200	2,170	499	2,669
11:30	Third count.	22,800	2,280	684	2,964

Generalization from these few observations is hardly warranted; yet there seems ground to suppose that pilocarpine, intravenously given, produces a prompt, moderate increase in the mononuclear cells, especially the lymphocytes, of the blood of the dog. Certainly no such extreme lymphocytosis takes place as Harvey noted

in the rabbit. A leukocytosis affecting the polymorphonuclear elements also made its appearance, but one cannot rule out the operation itself as sole cause of this. On the other hand, an absolute increase in the mononuclear cells, such as occurs here, is not a characteristic of the well-known leukocytosis due to operation. The dependence of the lymphocytosis on pilocarpine injection is indicated, furthermore, by those two instances in which repeated counts were made after the animal had been operated upon, but before the administration of the drug. In these counts the number of lymphocytes was found to be practically unvarying.

The direct cell-output by way of the lymph was now studied according to the method already described. It may be remarked in passing that lymphocytes are alone present in the dog's lymph in large number. (Delamere (8), Biedl and v. Decastello (9).)

Experiment IV.—Mongrel, male; wt. 8.2 kilo. Food was withheld from the animal for 18 hours previous to experiment. The duct was opened, and the lymph allowed to flow for 15 minutes before the first specimen was collected. It was pinkish, slightly opalescent, and at no time clotted in the cannula. When one specimen had been obtained 12 milligrams of pilocarpine nitrate were injected into the left, external jugular vein. The respirations became dyspnoëic for about one minute, after which they resumed their previous rhythm. The lymph flowed faster and was nearly colorless. Five specimens of it were collected, then a second injection of pilocarpine (11 milligrams) given, and two more specimens obtained. The cell-counts were made from the tubes in the order of their collection and 2 to 3 hours following it. (See Chart 1.)

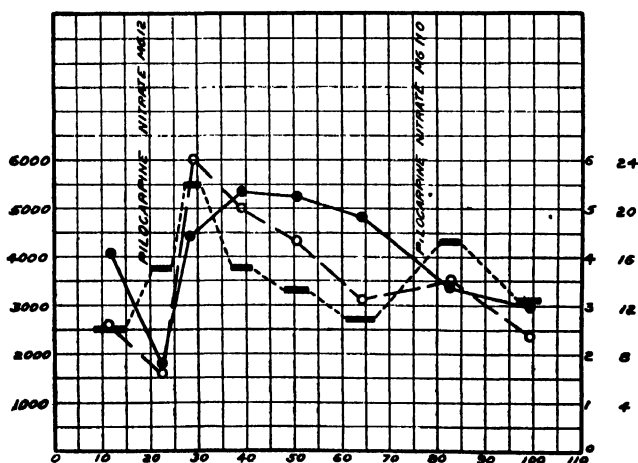


CHART 1.

An autopsy proved the animal to have been sound.

Experiment V.—Mongrel hound, male; wt. 9 kilo. Food was withheld for 26 hours previous to the experiment. The lymph was opalescent. A cannula was introduced into the duct after it had been ligated 5 minutes, and 26 minutes prior to the collection of the first tube. Slight clotting in the cannula necessitated twice in the two hours the use of a hooked wire to clean the bore. In one instance the flow was momentarily interfered with. This happened in an interval when lymph was not collected, and with the return of the flow 15 minutes were allowed to pass before another specimen was taken. The injection of the 5 milligrams of pilocarpine into the left, external jugular vein did not cause dyspnoea or movement. Two tubes of lymph were collected during the hour following. Cell-counts were made in the order of collection of specimens and 1½ hours after each had been obtained. (See Chart 2.)

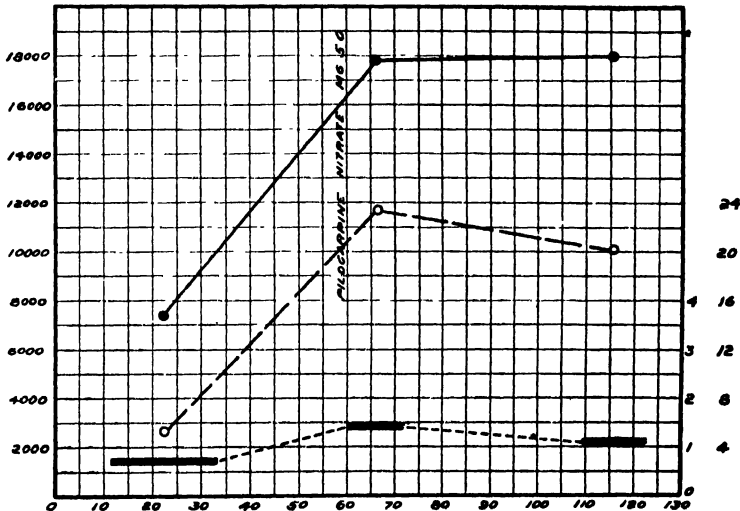


CHART 2.

The slow flow of lymph suggested the presence of an accessory thoracic duct. Accordingly, the duct proper was ligated before the animal was killed, and, with the aid of the natural injection, a search made for branches to the right side of the neck. None connected with the thoracic duct or receptaculum were found. The duct contained no clot and was patent.

Autopsy showed the animal to have been sound.

Experiment VI.—Male, collie; wt. 18.5 kilo. Food was withheld for 26 hours before operation. The thoracic duct was opened after 5 minutes ligation, and 20 minutes allowed to elapse before the collection of lymph was begun. Once in this interval the dog was partly roused by tweaking the skin, that the lymph-system might be flushed, through the quickened lymph-flow incident to struggle, of possible cell-accumulation in its channels. In the quiet following two tubes of lymph were taken, and after this 10 milligrams of pilocarpine nitrate

injected into the left, external jugular vein. The lymph, previously opalescent, became for 15 minutes quite milky, so that a fat ring developed in it on standing. The animal remained quiet and the character of its respirations did not change. Four more tubes were collected, then atropine sulphate, 0.6 milligrams, dissolved in a few minims of normal salt solution, was injected into the left subclavian vein, and two more tubes taken. The drug caused almost immediate cessation of bowel-noises, the flow of salivary secretion stopped, and the lymph slackened markedly in flow, and became clear and slightly blood-tinged. The subsequent injection of 15 milligrams of pilocarpine nitrate did not quicken its flow. There was no clotting in the cannula at any time. The tubes were counted in the order of their collection, and 1½ to 3 hours after it. (See Chart 3.)

At autopsy the animal was found to have been sound. The thoracic duct showed no branch leading to the right side of the neck.

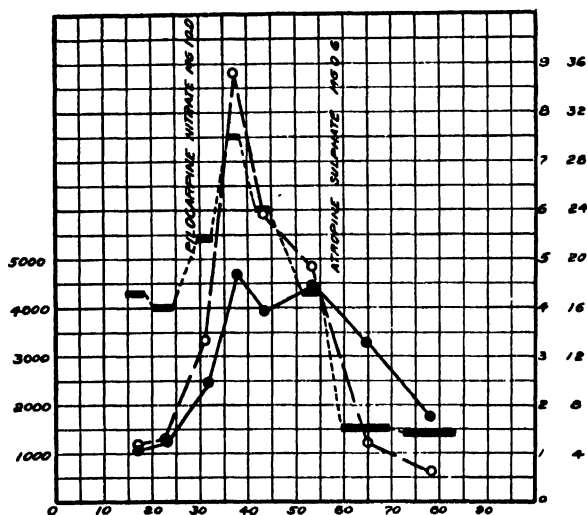


CHART 3.

The height above the base-line of the curve representing amount of lymph-flow indicates the number of cubic centimeters voided through the thoracic duct in a given time; and the black rectangles show the period required to collect the three cubic centimeters of lymph in each specimen. Thus the curve depicts in two ways the rapidity of lymph-flow.

The results of a first injection of pilocarpine in these three experiments are very similar. A well-defined increase in the number of white cells per cubic millimeter of lymph ("cell-concentration") is brought about, as also an increase in the total output of cells. The effect is fairly sustained, lasting one half to one hour. A quickening of the lymph-flow is also seen, but it is not so enduring.

One questions immediately whether the large cell-output is not a corollary to quickened lymph-flow. But comparison shows the two phenomena to lie in only a rough time-relation. Furthermore, as the following experiment demonstrates, pilocarpine will produce a profound increase of cell-output in a lymph-stream that varies little in flow. The effect of the drug is not always that of a lymphagogue (Heidenhain, Tschirwinsky (10), Spiro (11)).

Experiment VII.—Coach-dog, male; wt. 9.2 kilo. The animal was fed with lean beef $3\frac{1}{2}$ hours before operation. The duct was opened after 8 minutes ligation, and the lymph allowed to run for 19 minutes before the first specimen was collected. It was milky, and showed no tendency at any time to clot in the cannula. Two tubes were taken, and then 6 milligrams of pilocarpine nitrate injected into the left, external jugular vein. The animal continued quiet, and the breathing did not change in rhythm, yet during the next 20 minutes the content in fat of the chyle was much increased, as shown by a comparison of the fat-rings that formed in the tubes after they had stood for some hours. The fluid that ran later resembled thin chalk and water. Six specimens were collected, a second injection (5 milligrams) given, and three more tubes obtained. Toward the close of the experiment the breathing was slightly labored, and rhonchi could be heard. The tubes were counted in the order of their collection, and $2\frac{1}{2}$ to 4 hours after it. (See Chart 4.)

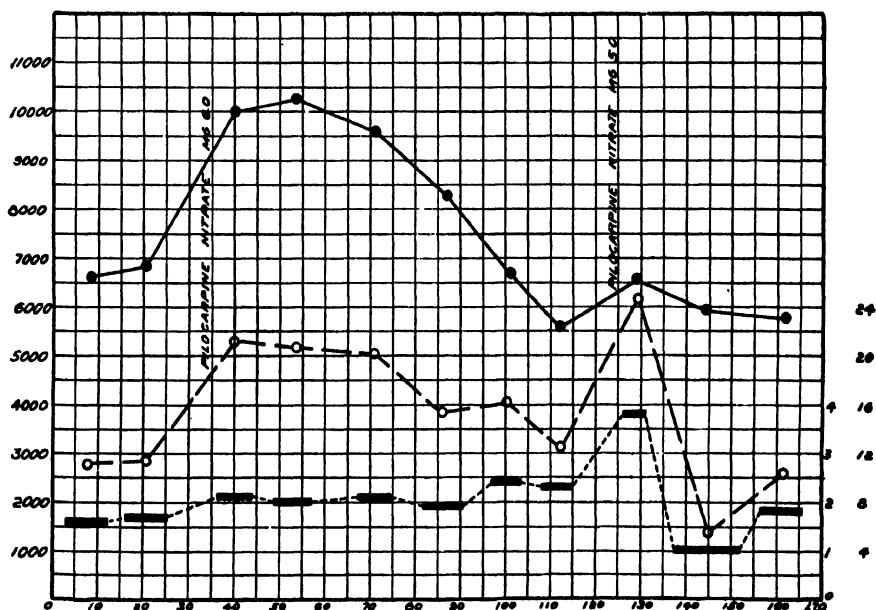


CHART 4.

The autopsy showed the animal to have been sound.

In this instance the effect on the cells of the first injection of pilocarpine was outspoken, despite the nearly constant lymph-flow. The cell-increase was prompt here, as in Experiments IV, V and VI. In all it took place in the ten minutes immediately after the injection.

The actual increase in cell-output is of interest as an indication of the extent to which contributions through the thoracic duct may be responsible for the pilocarpine lymphocytosis. The animals used for the work just presented moved voluntarily, or were induced to struggle, shortly before the experiment proper, to rule out that accumulation in the lymph-system of mature cells, which has been observed to occur in the quiet animal (Goodall and Paton (12), Rous). But the rush into the circulation of accumulated cells when pilocarpine acts on an animal previously quiet is to be reckoned with as an effect of the drug. The next experiment illustrates this.

Experiment VIII.—Collie, male; wt. 19 kilo. No food was given for 50 hours prior to the experiment. The animal was quiet for 1 hour before the collection of the first lymph-specimen which was taken 5 minutes after the opening of the thoracic duct. This had been 1 minute ligated. The slightly opalescent lymph showed no tendency to clot in the cannula. After two tubes of it had been obtained 10 milligrams of pilocarpine nitrate were injected into the left, external jugular vein. The breathing immediately became somewhat dyspnoic and remained so. Five tubes of lymph were taken, then a second injection of 10 milligrams given, and four more tubes obtained. Cell-estimations were made on the specimens in the order of their collection, and 2½ to 3½ hours after it. (See Chart 5.)

At autopsy a large mass of tape-worms was found in the small intestine. Otherwise the animal had been sound. The thoracic duct gave off no branch to the right side of the neck.

If one neglect the action of pilocarpine in changing the fluid content of the blood, a calculation is possible of the absolute increase in lymphocytes per cubic millimeter of blood which would have been caused by such an addition of cells as this.² The increased flow of lymph induced by the drug can hardly be supposed to act as a real diluent, since new lymph-production and active secretion from the salivary and other glands tend to drain the blood of fluid. On the basis that the dog, which weighed 19 kilo, had 7.7 per cent. of its

² After the administration of pilocarpine the bulk of white cells in the lymph is still one of lymphocytes.

weight in blood of a specific gravity of 1.055, one may assume 1,385 cubic centimeters as the total volume of blood. During the forty minutes following pilocarpine injection an average of 111,400,000 more white cells were given off through the thoracic duct in each five minutes than during the same period of quiet,—or a total excess over the normal outpouring of 891,000,000 in the

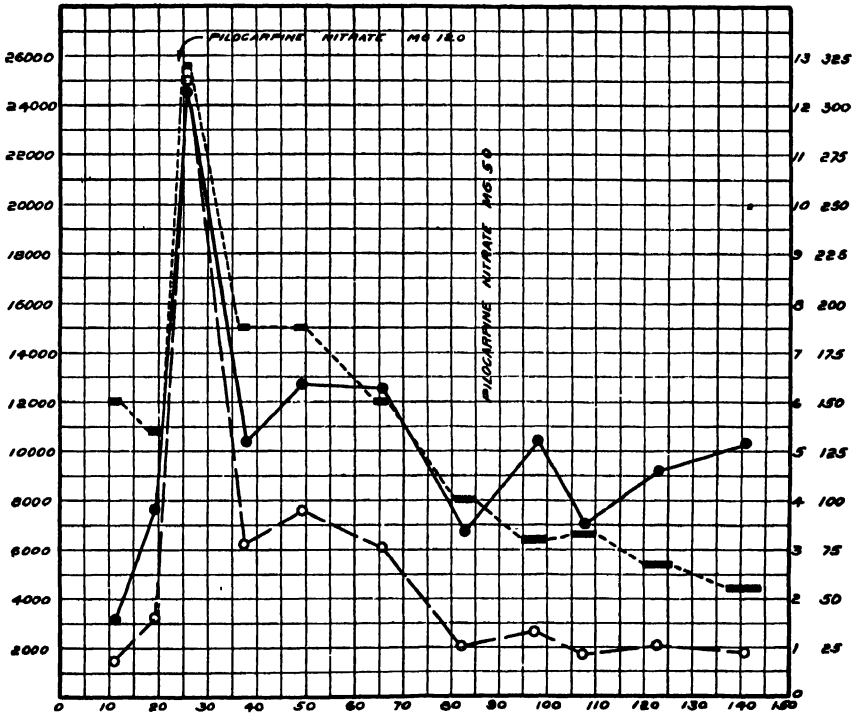


CHART 5.

forty minutes, a number sufficient to have furnished each cubic millimeter of blood with 643 lymphocytes over the normal supply.

The absolute increase in output of lymphocytes through the thoracic duct was much smaller in the other animals: enough in Experiment IV to have furnished 100 lymphocytes to each cubic millimeter of the dog's blood; in Experiment V sufficient for 382 per cubic millimeter; in Experiment VI enough in the short period of observation to give 84 extra cells per cubic millimeter; in Experiment VII enough for 146 extra cells per cubic millimeter. But

in Experiment VIII alone had the animal lain quiet as in Experiments I, II and III. In these three the increase in the circulating lymphocytes that took place in the first thirty to fifty minutes after pilocarpine injection was close to 1,000 per cubic millimeter. An output such as that obtained, despite the unfavorable condition of lymph-fistula, in Experiment VIII, would account for more than half of this lymphocytosis.

The effect of pilocarpine on cell-output through the thoracic duct, as here studied, is probably dependent on several factors:

1. *Increase in Lymph-Flow*.—Others have proved that pilocarpine often, though not always, acts as a lymphagogue. In a previous paper it has been shown that increase in lymph-flow alone,—the factor invoked by Ehrlich for the production of quickly appearing lymphocytosis,—exerts indeed a considerable influence to increase cell-output through the thoracic duct. When a condition of bodily quiet has allowed accumulation of cells in the lymph-system the number flushed out with a quickened lymph-stream may be large, as in Experiment VIII. Yet that the increased cell-output is but secondarily dependent on this factor has been made clear.

2. *Dyspæic Breathing*.—This is frequently induced by pilocarpine (Cushny (13)). In one only of the five experiments was it marked, though in a second it was briefly present. By its pumping action on the great lymph-channels of the trunk it tends to keep their contents in motion (Starling (14)), and would hinder in this way cell-accumulation.

3. *Contraction of Smooth Muscle*.—Pilocarpine contracts the lumen of the large lymph-vessels (Heinz (15)). Obviously a result of this narrowing is a very brief increase in the amount of lymph voided through the thoracic duct, and, as this is cell-containing, the total cell-output would also be briefly increased.

Harvey believes that contraction of the smooth muscle of lymph-glands and spleen is entirely responsible for the lymphocytosis he observed to follow pilocarpine injections in rabbits. He bases his conclusion principally on the fact that atropine prevents the occurrence of this lymphocytosis, whereas it does not hinder the occurrence of that which he found barium chloride to produce.

Whatever may be said of the effect of barium chloride on the

blood, it is certain that Harvey's atropine-pilocarpine experiments admit of a second interpretation as regards the process taking place in the lymph-system. For it is well-known (Spiro, Tschirwinsky) that atropine slows the lymph-flow strikingly, even though comparatively large doses of pilocarpine be given. Its action is in this way antagonistic to the increase of cell-output through the thoracic duct; for no matter if some force liberated cells from the lymph-glands, the stagnant current would prove but a poor medium for their transport. In illustration the effect of atropine in Experiment VI may be pointed out. Given shortly after pilocarpine, it immediately reduced lymph-flow and cell-output to less than they had been previous to the administration of either drug. The following experiment furnishes a further illustration:

Experiment IX.—Bull-dog, male; wt. 25 kilo. No food was allowed it for 24 hours previous to the experiment. The thoracic duct was opened after 3 minutes ligation, and the lymph ran free during 17 minutes before collection was begun. It was clear and at no time clotted in the cannula. Two specimens were taken to establish the facts of output, then 1.2 milligrams of atropine sulphate in a few minims of salt solution were injected into the left,

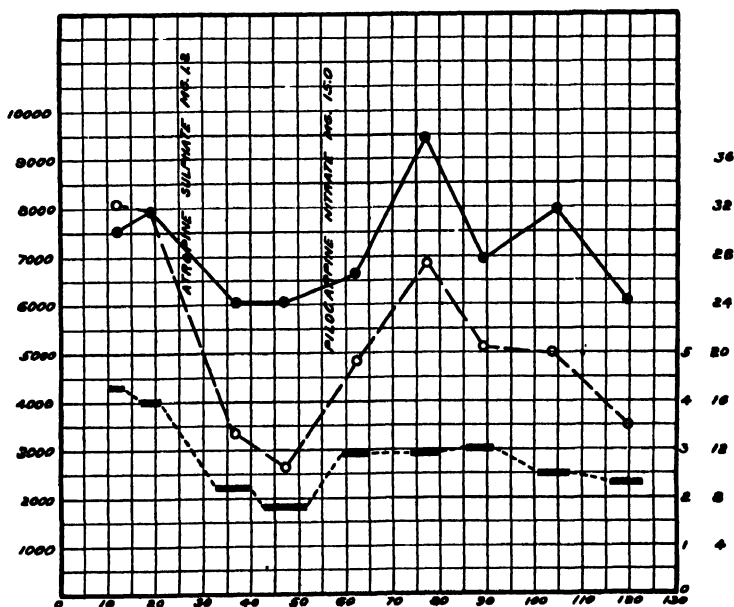


CHART 6.

external jugular vein, and, with decreased lymph-flow manifest, two more specimens obtained. The animal remained quiet. Fifteen milligrams of pilocarpine nitrate were now injected into the left, external jugular vein, causing a momentary flow of saliva, a few dyspnoëic movements of the chest, and twitching of the limbs, all of which ceased within the minute. No other changes in the animal's condition were noted. Five more tubes of lymph were obtained. Cell-counts were made in the order of tube-collection, and from 2 to 3 hours after it. (See Chart 6.)

Autopsy showed the animal to have been healthy. There were many tapeworms in the large intestine.

In this instance atropine reduced the lymph-output to one third its previous quantity, and cell-output by way of the lymph to much less than half. Pilocarpine raised the lymph- and cell-output again, but not to their rate previous to atropinization. It is impossible to say that the changes in cell-output are not wholly dependent on those of lymph-flow. Similarly, the profound fall in cell-output brought about in Experiment VI by atropine may be due to nothing else than lessened lymph-flow. To seek a further factor is unnecessary.

Yet some action of pilocarpine to further cell-output, other than those of increased lymph-flow and dyspnoëic breathing, is certainly present. Stimulation of the lymph-glands to productive activity cannot be responsible, since the increase in cell-output occurs practically at once. Were chemiotaxis a factor, as Gulland (16) believes, a second injection of pilocarpine ought to influence cell-output. But instances (Experiments IV, V, VI) of a second injection show it to have practically no effect. Contraction of smooth muscle must be further considered.

As has been demonstrated, the effect of atropine on the lymphocytosis of pilocarpine is neither for nor against its origin by smooth muscle contraction. That direct pressure (such as this contraction would bring about) may increase the cell-output of the lymph is highly probable, since the pressure exerted by a quickened lymph-flow will increase it. In this connection it is noteworthy that pilocarpine may render briefly chylous a lymph previously opalescent, or may, during a short period, increase the fat in one already milky (Experiments VI and VII). Either the absorption of fat from the intestine is for a brief time aided by the pilocarpine, or a larger proportion of intestinal lymph in the "mixed lymph" of the tho-

racic duct causes the latter to appear more chylous. The active movements of the intestine brought about by the drug, in association with Heidenhain's observation that much lymph may be squeezed out of the lacteals by direct pressure on a loop of the gut, makes this latter supposition probable; while the fact that the increase in fat of the "mixed lymph" appears abruptly and is transient speaks against the idea of an increase in absorption. Now, as is well known, the intestines and mesentery form the area of lymph-supply richest in lymph nodes and lymphoid tissue; and pressure changes in this area, taking place through contraction of the smooth muscle, may well be supposed to increase the output of white cells through the thoracic duct.

SUMMARY.

The intravenous injection of pilocarpine nitrate causes in the dog a rapid and considerable increase in the output of lymphocytes through the thoracic duct. The corresponding lymphocytosis induced by the drug in the blood of this animal is not profound, and increased cell-output with the lymph will explain a large part if not all of it.

Quickened lymph-flow and dyspnoëic breathing are accessory in the production of the large cell-output with the lymph, but it is mainly dependent on some undetermined element. The evidence points to the mechanical nature of this element. It is probably to be sought in direct pressure from contraction of smooth muscle, as suggested by Harvey, but his observation that atropine prevents the appearance of a lymphocytosis after pilocarpine cannot be quoted in proof because atropine much slows the lymph-flow, and thus decreases cell-output.

These findings are in accord with the theory that makes mechanical factors responsible for rapidly appearing lymphocytosis. They show that there are more such factors than has been supposed. Especially do they indicate that the contribution of cells through the thoracic duct may be important in the production of lymphocytosis, and is not, as is often asserted, subsidiary to direct migration into the blood of cells from spleen, bone-marrow and the lymph-glands.

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THE ENZYMES OF FIBRIN.¹

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The cells of inflammatory exudates contain two enzymes, one active in an alkaline, the other in an acid medium; the first belonging to the polynuclear leucocytes, and the second to the mononuclear phagocytes (1). The present work is an attempt to determine whether there is a resemblance between the enzymes of fibrin and those of inflammatory exudates in their behavior toward acid and alkali.

Fibrin has been long classed among the changed proteids, one of the chief characteristics of which is insolubility, and yet nearly a century ago it was observed by Arnold and by Berzélius (quoted by Arthus (2) and also by Dastre (3)) that fibrin was soluble in the presence of ammonium chloride. Later experimenters, especially Denis (4) in 1838, and Limbourg (5) in 1889, investigated many salts, including sodium chloride (Denis, Hammarsten (6), Green (7), Limbourg, Rulot (8)), sodium sulphate (Denis, Limbourg), potassium chloride, potassium iodide, sodium iodide, potassium bromide, ammonium nitrate, ammonium sulphate, magnesium sulphate (Limbourg), potassium nitrate (Denis, Zimmermann (9), Limbourg), calcium sulphate (Green), and aqueous solutions of sodium fluoride (Arthus, Dastre, Rulot). Fibrin was found to be soluble in solutions of nearly all these salts, though in some more readily than in others.

The most extended investigation of the action of acids was made by Fermi (10). He tested the effect of hydrochloric, sulphuric and nitric acid in five per cent. solutions, and of lactic, citric, acetic, butyric, oxalic, malic and formic acids in one per cent. solutions. All had a solvent effect, hydrochloric being strongest, and nitric, sulphuric, acetic and butyric weakest. Incidentally he allowed

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fibrin to stand in water, and found it dissolved, but no importance apparently was attached to this fact.

Alkali, in a five per cent. solution of sodium hydroxide, was tested by Deutschmann (11). This dissolved the fibrin of certain small animals, such as guinea-pigs, rats, mice, in less than an hour; of the larger animals, namely, dog, cat, pig, ox, and man, in several hours. In fact, unless the fibrin was previously heated to boiling, all these substances, neutral salts, acids, or alkalis, dissolved it with varying degrees of ease. Warmth was found to favor the action, while a low temperature retarded but did not prevent it. These facts suggest the action of an enzyme, but this conception has been years in developing; the earlier investigators accepted the phenomenon as a simple solution, the effect of a salt on fibrin.

The species of the animal was found to effect the ease of solution of the fibrin. Zimmermann, in 1846, reported that the fibrin of the ox and calf was insoluble, while that of man was soluble with difficulty. Deutschmann found the fibrin of the smaller animals more quickly soluble than that of the larger. Fermi, who experimented with the fibrin of the pig, sheep and horse found that the pig's fibrin dissolved most easily, the calf's with most difficulty, while fibrin of the horse and fibrin of the sheep were intermediate.

The view that a simple solution of fibrin occurs in the presence of various substances has had many supporters, even to within a few years. Evidence which weakened this theory was afforded by analysis of the resulting solution: two coagulable proteids were found by Green, one coagulating at about 56° C. and the second at about 60° C. Other investigators (Hasebroek (12), Herrmann (13), and Limbourg) found the coagulating point of the second product to be nearer 75° C., but agreed with Green upon the constant occurrence of these two products as a result of the solution of fibrin. Limbourg, in 1889, and Fermi, in 1891, found a third substance non-coagulable and giving the biuret reaction. Limbourg proved it to be a peptone. In 1894, Dastre again called attention to the formation of this third product, a soluble proteid, incoagulable by heat, and shown by the biuret test after the removal by heat coagulation of the two coagulable proteids previously mentioned. Dastre found this third product to consist of pro-peptones

and peptones, and its presence caused him to consider the disappearance of the fibrin a chemical alteration, a true digestion, and to suggest once more the possibility of a soluble ferment either derived from the blood and adhering to the fibrin, such as pepsin or trypsin (the two latter being the only two soluble proteolytic ferments generally known at that time) or introduced into it by putrefactive organisms.

Many believed that the decomposition of fibrin was due to the putrefactive organisms, but this possibility was disproved by Green (9), who conducted his experiments at a temperature only a little above freezing, and in a ten per cent. sodium chloride solution, too strong a solution to favor the putrefactive organism; and by Dastre, who took every precaution to exclude microorganisms and used a fifteen per cent. sodium chloride solution and a two per cent. sodium fluoride solution. Other observers have prevented development of microorganisms by a variety of substances, such as chloroform, alcohol, phenol, ether, thymol, hydrocyanic acid and toluol.

Putrefactive organisms being excluded, a possible enzyme was still to be found. Dastre discredits the idea that pepsin is present in amount sufficient to digest fibrin, for otherwise pepsin could always be found in considerable quantities in the blood, and its presence cannot be demonstrated. Moreover, pepsin must act in an acid medium; two per cent. sodium hydroxide destroys pepsin, but dissolves fibrin. Neither, Dastre adds, can it be trypsin, because trypsin breaks down fibrin into true peptones, with formation of tyrosin; but no matter how long fibrin remains in salt solution, no tyrosin is produced.

Though Friedrich Müller (14) had demonstrated in 1888 the presence of a proteolytic enzyme in the leucocytes, its part in the solution of fibrin was not investigated until Rulot proved in 1904 that the digestion of fibrin was caused by leucocytes imprisoned in the meshes of fibrin. Rulot's work is particularly satisfactory because it is so conducted as to control the presence or absence of leucocytes, and to measure the results of digestion, not macroscopically, but more accurately, by the Kjeldahl determination of nitrogen. In order to get pure fibrin, free from leucocytes, he used two methods. In one he prevented the clotting of the blood

by the addition of sufficient concentrated salt solution to make the whole volume of blood a four per cent. solution of sodium chloride. The blood was allowed to settle and the supernatant plasma was pipetted off and filtered. When diluted to about four times its volume with water at about 50° C., fibrin was formed. In the second method of obtaining pure fibrin coagulation was prevented by injecting a solution of pro-peptone into the external jugular vein; from the plasma after centrifugalization and filtration, fibrin was formed by a current of carbon dioxide. This pure fibrin was very nearly insoluble in saline solutions, but the same fibrin taken from unfiltered plasma and having added to it the superficial layer of corpuscles, containing great numbers of leucocytes, digested rapidly, with the formation of peptones and pro-peptones, which were rarely found after the very slight solution taking place with pure fibrin.

Rulot's work has so well established the fact that disappearance of fibrin in salt solutions is due to the action of a proteolytic enzyme present in the leucocytes, that the present work is devoted to a study of the effect of acid and alkali on the digestion of fibrin, with a view to testing the identity of the enzyme or enzymes present with those already demonstrated in the leucocytes of inflammatory exudates.

Methods.—The methods used have been two. First, solution of fibrin, observed by a macroscopic examination of fibrin suspended in acetic acid and in sodium carbonate of concentration from 0.1 per cent. to five per cent., and in neutral solutions. Macroscopic evidences of the progress of solution are the disappearance of the fibrin, the turbidity and final clearing of the solution, though the last two factors are of slight importance. The time required for the solution of fibrin is a rough indication of the activity of self-digestion. The presence of peptones or albumoses in solution was shown by the buiret test, after the removal of the coagulable proteids by precipitation with trichlor-acetic acid and filtration, the alkaline solutions being neutralized before the addition of the acid.

The second method, which is far more reliable, is the determination by the Kjeldahl method of the nitrogen in soluble incoagulable substances formed from fibrin. This method was used first to

measure the degree of autolysis of fibrin alone. The small figures representing nitrogen in incoagulable substances obtained after solution of fibrin were apparently due to the limited amount of proteid (fibrin) upon which the enzyme might act. To gain an idea of what the enzyme was capable, it was thought desirable to furnish additional proteid, such as heated serum, which a proteolytic enzyme could attack. The figures obtained were much greater than with fibrin alone.

Fibrin was prepared in the ordinary way, by whipping freshly drawn blood. The fibrin was then washed with running water for several hours and dried by squeezing in sterile gauze. For the macroscopic study of solution, 0.1 gram of fibrin was added to each test-tube in which the total volume of liquid was five cubic centimeters, and incubated for four days at a temperature of 37° C.

For the experiments in which digestion was measured by the Kjeldahl method, 0.3 gram of fibrin was added to flasks, each of which contained a total volume of twenty-five cubic centimeters, five cubic centimeters of heated serum, acetic acid or sodium carbonate varying from 0.2 to five per cent. and sodium chloride solution (0.85 per cent.) to make up the required volume. These flasks were then incubated for five days at 37° C.; the contents was coagulated by heating in the water bath, after addition of magnesium sulphate and acidifying with a one per cent. acetic acid solution. The contents of the flask were neutralized by a one per cent. sodium hydroxide solution, and brought to the boiling point over the flame. The incoagulable proteid was then filtered directly into Kjeldahl flasks, the coagulum being washed repeatedly, digested with sulphuric acid and distilled. The amount of nitrogen is expressed in cubic centimeters of $\frac{1}{10}$ N sulphuric acid.

Fibrin dissolved to a considerable degree in all strengths of alkali (sodium carbonate) from 0.1 to five per cent., the biuret reaction being more marked after solution in the lower strengths from 0.1 to 0.5 per cent.

In acetic acid, fibrin dissolved somewhat in strengths from 0.1 to four per cent., very little, if at all, in a five per cent. solution. The biuret reaction after coagulation with trichlor-acetic acid, was more marked in the weakest acid, that is, from 0.1 to 0.4 per cent.

The strongest biuret reactions were obtained when fibrin was incubated in neutral media and in 0.1 and 0.2 per cent. solutions of carbonate. The biuret test was not always strongest when the disappearance of fibrin had been greatest, though this relation existed as a rule. Observation of solution, with the biuret test as a measure of incoagulable proteid, was very unsatisfactory. The nitrogen determinations by the Kjeldahl method gave far more decisive results.

In proof of the fact that the enzyme of fibrin acts on foreign proteid as well as on fibrin itself after incubation at 37° C. during five days, the following experiments are given, the amount of nitrogen of the incoagulable proteid being expressed in cubic centimeters of $\frac{1}{10}$ N sulphuric acid.

TABLE I.

The action of acid and alkali on fibrin alone, and on fibrin + heated serum.

	Experiment.	Control.	Acetic acid.			Neutral.	Sodium carbonate.		
			5 per cent.	2 per cent.	0.2 per cent.		0.2 per cent.	2 per cent.	5 per cent.
Fibrin ... }	a.	0.35	1.35	1.6	2.55	2.9	—	—	—
	b.	0.9	—	—	—	5.45	2.6	3.5	3.4
Fibrin + serum.. }	a.	1.9	3.45	6.7	3.7	15.7	—	—	—
	b.	2.9	—	—	—	19.6	25.6	9.25	11.1

Figures obtained when fibrin undergoes autolysis are much less than those obtained with fibrin and serum; the excess is referable to decomposition of proteid of the serum.

TABLE II.

Fibrin + heated serum, in presence of sodium carbonate.

Experiment.	Control.	Neutral.	Sodium carbonate.				
			0.2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	5 per cent.
1	2.00	16.35	14.1	2.8	3.25	—	—
2	1.95	15.70	1.95	2.6	3.5	—	—
3	2.90	19.60	25.6	—	—	9.25	11.1
4	2.35	18.65	20.35	4.8	4.2	12.15	7.85
5	2.30	11.3	12.6	4.2	4.25	5.2	8.05
6	1.95	12.7	5.7	5.2	6.3	—	—
7	2.05	9.65	2.55	2.9	3.9	6.25	7.55

Digestion Caused by Fibrin in the Presence of an Alkaline Medium.—The following table shows the action of fibrin on heated serum, in the presence of an alkaline medium.

These nitrogen determinations show that in whatever strength of carbonate digestion proceeds, there is always some production of an incoagulable proteid. Experiments 4 and 5 were performed with the same fibrin and serum; in Experiment 5, digestion was stopped at the end of twenty-four hours, while in Experiment 4 it was allowed to continue for five days, the period allowed for digestion in the other experiments. Judged by the macroscopic disappearance of the fibrin and by the clearness of the solution there was about as much proteolysis at the end of twenty-four hours as at the end of five days, while the Kjeldahl method showed a decided increase after the longer period.

While in the majority of experiments digestion in 0.2 per cent. carbonate has been greater or approximately equal to that in neutral solution, in several experiments no digestion occurs in the 0.2 per cent. carbonate, though digestion in neutral solution has been active. It has not been possible to explain these exceptions. The same divergence is found in the following experiments, in which (save in the last) washed polynuclear leucocytes from sterile pus produced by repeated intrapleural injection of turpentine, have acted upon heated blood serum. In the last experiment leucocytes were obtained from an abscess produced by subcutaneous injection of turpentine.

TABLE III.

Cells from purulent exudates + heated serum.

Control.	Neutral solution.	0.2 per cent. carbonate.
2.6	15.00	7.75
2.2	13.95	4.8
2.65	14.95	13.75
3.2	22.75	27.95
3.55	19.35	21.35
2.9	15.95	20.9
3.45	16.25	22.45

The foregoing experiments with fibrin (Tables I and II) demonstrate the presence of an enzyme which acts either upon fibrin or upon foreign proteids, such as those of heated blood serum, in the

presence of a neutral or very weakly alkaline reaction. A strength of alkali greater than 0.2 per cent. sodium carbonate is unfavorable to the action of this enzyme and inhibits it. By increasing the strength of alkali to from two to three per cent. sodium carbonate there is an increase of incoagulable nitrogen-containing substances which doubtless are not referable to enzyme action, and perhaps are formed by action of the stronger alkali upon proteid. Incomplete coagulation in the presence of a large quantity of sodium carbonate, which is accurately neutralized with difficulty, may explain part of the increase of nitrogen obtained in the presence of the higher percentages of alkali.

Digestion in the Presence of Acid.—The amount of nitrogen produced by autolysis of fibrin in the presence of acetic acid is given in the following table.

TABLE IV.
Autolysis of fibrin, in presence of acetic acid.

Experiment.	Control.	Neutral.	Acetic acid.				
			0.2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	5 per cent.
1	0.4	—	2.05	2.35	2.4	—	—
2	0.75	4.2	2.65	2.05	1.95	—	—
3	0.35	2.9	2.55	—	—	1.6	1.35

The table does not give evidence that a ferment capable of digesting in the presence of weak acid is present in any considerable amount. In two experiments solution with acid was greatest in the presence of 0.2 per cent. acetic acid and may have been referable to enzyme action. The figures obtained are so small that definite conclusions are not possible.

TABLE V.
Fibrin + heated serum, in the presence of acetic acid.

Control.	Neutral.	Acetic acid.					
		0.2 per cent.	0.5 per cent.	1.0 per cent.	2.0 per cent.	3.5 per cent.	5.0 per cent.
1.6	23.00	9.5	—	12.2	13.65	—	8.55
2.35	18.65	5.8	7.75	7.30	8.15	—	4.50
1.95	15.70	3.7	—	—	6.7	—	3.45
1.95	18.8	3.95	—	4.6	5.75	—	—
2.3	11.3	4.0	3.95	3.95	4.05	—	3.55
1.95	12.7	7.7	6.65	5.3	4.9	—	3.5
2.25	21.65	8.2	10.95	—	10.00	8.75	7.15

In the following experiments fibrin was allowed to act upon heated serum in the presence of acid. Since the figures thus obtained are much greater than those with autolysis alone, it must be assumed that the proteid of the serum has been decomposed.

The mononuclear cells of an inflammatory exudate contain an enzyme which digests proteid in the presence of weak acid and fails to digest in the presence of an alkaline reaction. The following experiment, in which fibrin was obtained from the pleural cavity six days after the injection of turpentine, shows the behavior of this enzyme in the presence of various strengths of sodium carbonate and of acetic acid; at this time the enzyme of the polynuclear leucocytes has disappeared from the fibrinous exudate (1).

TABLE VI.

Action of fibrin of an inflammatory exudate on heated blood serum in presence of acid and alkali.

Control.	Acetic acid.					Neutral.	Sodium carbonate		
	5.0 per cent.	3.5 per cent.	2.0 per cent.	0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.	1 per cent.
2.0	5.05	4.85	3.15	8.45	9.05	17.2	4.05	4.5	4.7

Enzyme digesting in alkali (leucoprotease) is present (Table VI), if at all, in very small amount, but the enzyme (lymphoprotease), which digests in acid, is active. The experiment suggests that this enzyme digests well in the presence of a neutral medium. This fact may explain some of the discrepancies previously noted (Table II).

Table VI shows that the enzyme of fibrin of the inflammatory exudate, which is active in 0.2 and 0.5 per cent. of acetic acid, is almost completely inhibited by two per cent. acid. The higher figures obtained with greater strength of acid are probably referable to direct action of the acid upon the proteid used, or to incomplete coagulation as the result of inaccurate neutralization. In all of the experiments with fibrin of the blood figures obtained with 0.2 per cent. of acid are considerably greater than those of the control. That enzyme of fibrin which acts in an alkaline medium is identical, it has been shown, with leucoprotease obtained from

plynuclear leucocytes of an inflammatory exudate. But since leucoprotease when obtained by treatment of leucocytes with alcohol and ether fails to cause digestion in the presence of acid, that digestion which occurs with fibrin of blood in the presence of acid is explained by assuming the presence of a second enzyme.

When fibrin of blood acts on heated serum in the presence of acetic acid, increasing the strength of the acid produces a corresponding increase in the amount of nitrogen (Table V), the maximum, as a rule, being reached with a two per cent. concentration of acetic acid. Further increase causes an inhibition of formation of incoagulable nitrogen containing substances. This maximum of digestion in two per cent. acid has not been observed with the enzyme of the inflammatory exudate, and is not explained by the available data.

To test further whether the amount of proteid disintegration obtained was due to the enzyme action, or merely to the action of the acid on the proteid, the effect of unheated fibrin and of fibrin heated to 100° C. upon heated serum was tested, and for further comparison the action of acid alone on heated serum.

TABLE VII.

Control experiments.

Experiment.		Acetic acid					
		0.2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	3.5 per cent.	5 per cent.
<i>a</i>	Unheated fibrin + heated serum.	3.95		4.6	5.75		
	Heated " + " "	2.5		3.65	3.45		
	Heated serum.	1.65			1.65		2.4
<i>b</i>	Unheated fibrin + heated serum.	8.2	10.95		10.00	8.75	7.15
	Heated " + " "	2.8	2.8		3.7	3.45	4.1
	Heated serum.	2.9	2.95		4.15	4.2	4.3

The experiments with the heated and unheated fibrin show a greater production of incoagulable nitrogen when the enzyme has not been destroyed by heating, while those with heated serum suggest that the acid itself in strength from two to five per cent. causes disintegration of fibrin and of proteid, increasing with the strength of the acid.

Conclusions.—(a) There is present in fibrin an enzyme which acts in a neutral and slightly better in an alkaline medium, thus resembling the enzyme present in the polynuclear leucocytes obtained from an inflammatory exudate. This enzyme acts not only on fibrin, causing autolysis, but upon foreign proteid (coagulated blood serum) as well. The action of this enzyme is inhibited by increasing the strength of the alkali above 0.2 per cent. sodium carbonate.

(b) Fibrin contains an enzyme which acts in the presence of a weak acid. This enzyme acts upon foreign proteid as well as upon fibrin itself and is probably identical with the similar enzyme which occurs in the large mononuclear cells of an inflammatory exudate.

I wish to acknowledge my indebtedness to Dr. Opie for suggesting this problem to me, and for his assistance and oversight during the course of the work.

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THE EFFECT OF INJECTED LEUCOCYTES UPON THE DEVELOPMENT OF A TUBERCULOUS LESION.*

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The purpose of the experiments which will be described has been to determine the effect of injected leucocytes upon the development of a tuberculous lesion. For the experiments the dog, which is somewhat insusceptible to tuberculosis has been selected for two reasons. Preceding studies have demonstrated methods by which it is possible to obtain sterile leucocytes in great quantity almost wholly free from the inflammatory irritant which has been used to cause their accumulation. Of equal importance for the purpose of the experiments is the fact that the development of the tuberculous lesions produced by injection of tubercle bacilli into the pleural cavity of the dog can be followed with considerable accuracy by percussion of the animal's chest.

The insusceptibility of the dog to tuberculosis has been exaggerated and its apparent immunity to the disease is doubtless dependent in part upon the fact that its habits do not expose it to infection. Freedom from spontaneous infection is not an accurate index of susceptibility, for the guinea-pig, in which tuberculosis develops with great readiness, is rarely subjected to spontaneous infection. Spontaneous tuberculosis in dogs has been studied especially by Jensen (twenty-eight cases), Cadiot (forty cases) and Eber¹ (eleven cases). The lungs are the primary seat of infection in a large proportion of the cases. The pleura and mediastinal lymphatic glands are implicated. Jensen has described the sarcoma-like appearance of tuberculous tissue in the dog and thinks that tuberculosis of various organs in this animal closely resembles the same lesion in cattle.

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¹ Eber, Lubarsch and Ostertags *Ergebnisse der allg. Path.*, 1897, iv, 859.

Injection of a suspension of tubercle bacilli into the pleural cavity of dogs causes tuberculosis which is almost constantly fatal. Fluid accumulates in the cavity; flat nodules of grayish white tuberculosis tissue are formed upon the pleura of the chest wall and diaphragm and occasionally upon the surface of the lungs. The lesion is bilat-

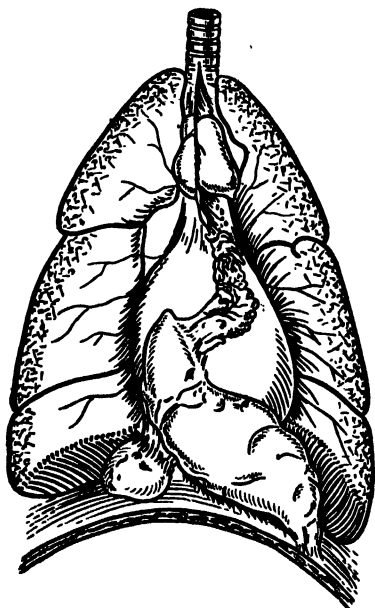


FIG. 1.

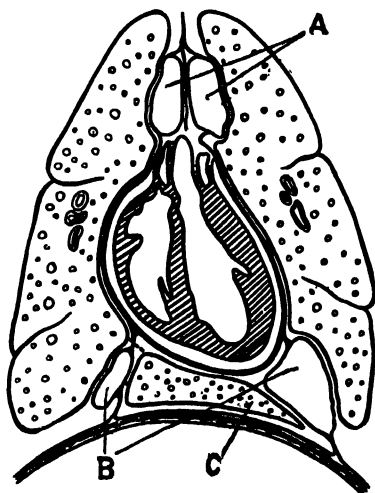


FIG. 2.

FIG. 1. Diagram to accompany Experiment 10, showing distribution of tuberculous tissue after inoculation of the right pleural cavity with tubercle bacilli. Tuberculous tissue occupies the mediastinum and subpericardial membranes; the substernal lymphatic glands are enlarged and tuberculous.

FIG. 2. Lesion represented by the preceding figure in section showing the membranes which extend from the pericardium to diaphragm and partially enclose a cavity into which fits a lobe of the right lung (C). B, tuberculous tissue in subpericardial membranes; A, enlarged substernal lymphatic glands.

eral and fluid accumulates in both the right and left pleural cavities, but the disease usually progresses more rapidly in the cavity which has received the injection. Rounded masses of firm gray-white tissue appear in the mediastinum (Fig. 1) and when the lesion is advanced may grow together, forming a continuous mass extending the whole length of the sternum. The tissue is at first succulent

grayish white and homogeneous, having an appearance suggesting sarcoma, but later becomes opaque and yellow where caseation occurs. Similar masses of tuberculosis tissue (Fig. 2), often continuous with those in the mediastinum, occupy the membranes which in the dog extend from each side of the pericardium to the diaphragm and enclose a cavity; into this cavity fits a lobe of the right lung. The mediastinal lymphatic glands (Figs. 1 and 2), situated behind the upper end of the sternum, quickly enlarge, become hard and undergo caseation.

Dissemination of tubercle bacilli occurs at an early period of the disease and ten days after inoculation tubercles may be found in the liver. After many weeks tubercles may be found in the lungs, spleen and kidneys. Extension by way of the lymphatic system occurs rapidly and evidence of tuberculosis appears successively in the retropleural glands just above the diaphragm, in glands near the duodenal part of the pancreas and in the retroperitoneal glands which are near the adrenals.

Death frequently occurs as the result of broncho-pneumonia due to secondary infection. After several weeks the animal may cough and there is abundant purulent discharge from the nose. Associated with this condition conjunctivitis and ulceration of the cornea may occur.

The experiments which have been described have been possible only because the course of the disease can be followed during life by percussion of the chest. In the normal animal standing in its usual position there is relative dullness two or three centimeters to the right of the median line caused by projection of the heart to the right. The median line of the animal is marked and the upper limits of relative and absolute dullness accurately measured immediately behind the fore-leg; figures thus obtained are accurate within less than half a centimeter and afford the only available means of measuring during life changes in the fluid and solid contents of the chest.

After injection of tubercle bacilli increase of relative dullness over the ventral part of the right chest is evident within one or two days and this impaired dullness increases continuously. Usually within a week or ten days absolute dullness makes its appearance.

Changes in the extent of dullness on percussion are referable in part to accumulation of fluid, in part to solid tuberculosis masses in the mediastinum and adjacent membranes. Observations made by puncture of the animal's chest and by autopsy indicate that absolute dullness is caused by the presence of fluid. Not infrequently in inoculated animals absolute dullness disappears although increased relative dullness persists and the disease continues with undiminished severity. In such instances disappearance of absolute dullness is doubtless due to absorption of pleural effusion.

The following experiments illustrate the effect on dogs of intrapleural injection of the strain of tubercle bacillus employed in the greater number of the experiments which will be described. The organism had the characters of the human type of tubercle bacillus and was of only moderate virulence, killing guinea-pigs by intraperitoneal injection after from three to four weeks; it did not kill rabbits after injection into the peritoneal cavity, but caused their death six or seven weeks after intrapleural inoculation.

EXPERIMENT 1.—Dog, wt. 4,150 grm. Into the right pleural cavity was injected 0.5 c.c. of a suspension of *B. tuberculosis*. Relative and absolute dullness over the right side of the chest gradually increased so that at the end of about a month relative dullness measured 8.5 cm. and absolute dullness 6.6 cm. Below the skin at the point of injection a nodular mass fixed to the chest wall made its appearance two weeks after inoculation, increased in size, and opened spontaneously; it remained as an open wound until death which occurred with increasing emaciation at the end of 50 days after inoculation.

Autopsy.—Each pleural cavity contains about 150 c.c. of almost clear yellow fluid which on standing forms a transparent coagulum. Upon both visceral and parietal pleuræ are flat, yellowish-white nodules. Masses of hard, partly caseous tuberculous tissue occur in the mediastinum and in the membranes extending from pericardium to diaphragm. The substernal lymphatic glands are greatly enlarged and caseous. The liver contains an immense number of miliary tubercles which occupy about one half the area of the section prepared for microscopic examination. The spleen and kidney contain tubercles in small number.

EXPERIMENT 2.—Dog, wt. 4,850 grm. Into the right pleural cavity was injected 1 c.c. of a suspension of *B. tuberculosis*. Dullness over the ventral part of the thorax on the right side increased so that at the end of two weeks the upper level of relative dullness was 7.6 cm. from the mid line; of absolute dullness, 3 cm. During this time the body weight had been maintained. The animal became thin and death occurred at the end of 20 days after the inoculation.

Autopsy.—The right pleural cavity contains 75 c.c. of almost clear yellow fluid; the left cavity contains 80 c.c. Upon the surface of both lungs are flat gray-white projections usually less than 1 mm. across. The mediastinum which is thickened contains large masses of newly formed hard grayish white tissue;

a similar mass is in contact with the diaphragm. The lymphatic glands below the cephalic end of the sternum are greatly enlarged and caseous. The liver contains an immense number of tubercles which occupy at least a third of the section for microscopic examination. The spleen contains tubercles; none are found in the kidneys.

Inoculation with 0.5 c.c. of a suspension of tubercle bacilli caused death in fifty days whereas twice this amount of the same suspension was more quickly fatal.

The leucocytes used for injection have been obtained from dogs by repeated injection of turpentine into the pleural cavity. One or two cubic centimeters of turpentine have been injected into the right pleural cavity; when after three days the resulting inflammatory exudate has reached a maximum a second similar injection is made. Fluid continues to accumulate and may be serous, seropurulent or purulent. Aspiration of this fluid is followed by accumulation of purulent exudate of which a third of the volume may be leucocytes.

Leucocytes obtained one or two days either after injection of turpentine or after aspiration are separated by centrifugalization from the serum of the exudate and twice washed by centrifugalization with normal salt solution. The leucocytes after removal of the overlying salt solution readily pass through the coarse needle of a syringe. The quantities injected represent volumes of leucocytes packed together by centrifugalization.

A coarse needle with blunt beveled point and with an opening at the side a short distance from the end has proved convenient for intrapleural injection. The skin is punctured with a sharp instrument and the needle of the syringe is inserted obliquely in such position that the beveled surface of the end is parallel with the chest wall.

Washed leucocytes obtained by the method which has been described cause a readily recognizable reaction when introduced into the right pleural cavity of a normal dog. Ten cubic centimeters of these cells cause an accumulation of fluid which is indicated by a broad area of relative and usually of absolute dullness over the dependent part of the cavity. This increased dullness reaches a maximum on the day following injection and rapidly subsides, disappearing after three or four days. The quantity of fluid which accumulates (indicated by the amount of dullness) and the dura-

tion of the reaction increases with the quantity of injected leucocytes.

The pleural cavity is not permanently altered by the reaction which occurs. In an animal which had received four injections of leucocytes (10 to 25 c.c.) at intervals of about one week the pleural cavities were found to be normal and the mediastinum and adjacent membranes delicate.

Series A.—The effect of injections of leucocytes upon thoracic dullness increased by inoculation with Bacillus tuberculosis.

One half cubic centimeter of a suspension of *Bacillus tuberculosis* was injected into the right pleural cavity of six dogs. Dullness on percussion over the right side of the thorax underwent an increase, exhibiting in different animals considerable variation in rapidity. Two animals (weighing respectively 5,050 and 7,250 grm.) in which the disease was allowed to pursue its course served as control. At the end of seven or eight days, when relative dullness was much increased and absolute dullness had made its appearance in all of the inoculated animals, leucocytes in quantities from twelve to twenty-five cubic centimeters were injected into the right pleural cavities of the remaining four dogs; the injections were repeated at intervals of about one week.

For extraneous reasons it was found necessary to discontinue injection of leucocytes at the end of one month after inoculation; at this time the area of dullness had in the four injected animals diminished considerably and was not much greater than that present before the onset of tuberculosis. It was thought possible that recovery might follow but examination of the chest on the fortieth day of the disease showed that in two animals there was increase of relative and reappearance of absolute dullness.

EXPERIMENT 3.—Dog, wt. 5,450 grm. The animal received three intrapleural injections of leucocytes; changes in thoracic dullness are indicated below. At the end of a week a small nodule was found below the skin at the point at which the inoculating needle had been inserted; ten days later leucocytes were injected into the nodule. It became smaller but finally broke upon the surface and remained as a discharging ulcer until death. Death occurred 57 days after inoculation.

Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	2.7	Inoculated with tubercle bacilli.
9	3.0	3.9	14 c.c. leucocytes injected.
10	4.7	6.6	

12	—	4.3	
19	—	3.4	12 c.c leucocytes injected.
20	—	3.4	
27	—	5.3	10 c.c. leucocytes injected.
28	—	4.5	
29	—	3.8	
40	4.6	8.1	
58			Died.

Autopsy.—The pleural cavities are each distended with several hundred cubic centimeters of turbid fluid which compresses the lungs. The mediastinum and subpericardial membranes which are thickened and opaque contain large confluent gray white masses with a maximum thickness of 0.5 cm. The mediastinum is pouched to the right and much crinkled. Parietal and pulmonary pleuræ are thickened and opaque. The substernal lymphatic glands are moderately enlarged and partially caseous. The liver is large and contains an immense number of tubercles. An occasional tubercle is found in the lungs.

EXPERIMENT 4.—Dog, wt. 6,850 grm. The animal was inoculated into the right pleural cavity with 0.5 c.c. of a suspension of *B. tuberculosis*. A nodule formed in the skin at the point of injection. The progress of the disease during which leucocytes were injected three times is shown by the following table. Death occurred after 68 days.

Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	4.0	Inoculated with <i>B. tuberculosis</i> .
9	4.0	5.6	25 c.c. leucocytes injected.
10	3.0	5.6	
12	—	3.7	
19	—	3.7	11 c.c. leucocytes injected.
20	3.3	7.0	
22	—	4.8	
27	—	4.8	10 c.c. leucocytes injected.
28	—	4.6	
40	—	4.6	
69			Died.

Autopsy.—The pleural cavities contain a large quantity of fluid which compresses the lungs. The mediastinum contains masses of newly formed tissue which is dense and fibrous and contains caseous patches. Masses of similar tissue occur in the membranes which extend from pericardium to diaphragm. The lungs are atelectatic and contain upon their surfaces and in their substance numerous tubercles. About one third of a section of liver consists of tuberculous tissue.

EXPERIMENT 5.—Dog, wt. 6,750 grm. The animal received into the right pleural cavity 0.5 c.c. of a suspension of tubercle bacilli. A nodule appeared at the point of inoculation about a week later. Leucocytes were injected into the nodule which subsequently diminished much in size. The animal received four injections of leucocytes into the pleural cavity, it became emaciated and died at the end of 89 days.

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Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	2.5	Inoculated with <i>B. tuberculosis</i> . 12 c.c. leucocytes injected.
9	4.9	7.4	
10	2.0	3.9	
12	—	3.1	
19	—	4.1	22 c.c. leucocytes injected.
20	2.0	5.0	
22	—	4.2	
27	—	4.6	
28	—	4.9	10 c.c. leucocytes injected.
33	—	4.0	
40	2.8	6.8	10 c.c. leucocytes injected.
90			
			Died.

Autopsy.—Pleural cavities contain a large amount of fluid. The pleura is thickened and opaque and on its surface in places is a thin layer of fibrin. The

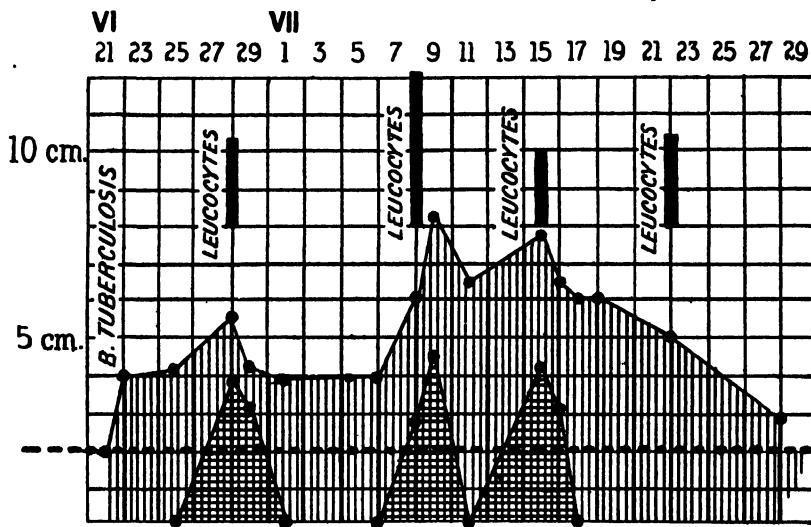


CHART I.—The progress of the disease in Experiment 6 and in subsequent experiments has been depicted by a chart which indicates the amount of absolute and relative thoracic dullness measured to the right of the mid line. Injection of leucocytes is indicated by heavy black perpendicular lines whose length (one square equals 5 c.c.) represents the quantity of cells injected. Relative dullness is represented by the lightly shaded zone; absolute dullness by the heavily shaded areas. In the normal animal there is no absolute dullness over the thorax to the right of the mid line but since projection of the heart to the right causes relative dullness two or three centimeters beyond the mid line a normal base line (dotted horizontal line) of relative dullness has been drawn for each animal. Weight is indicated by a dotted line at the upper part of each chart.

mediastinum is free from tuberculous masses save above the diaphragm where there is a mass of fibrous and caseous tissue; in the membranes which extend from pericardium to diaphragm are similar masses, that on the right being the larger. The mediastinal lymphatic glands are moderately enlarged and consist of caseous material surrounded by a fibrous capsule. The lungs and liver contain tubercles in immense number. In the liver they are surrounded by a thin fibrous capsule.

EXPERIMENT 6.—Dog, wt. 4,750 grm. Seven days after inoculation with *B. tuberculosis* as a time when relative dullness over the right chest had increased and absolute dullness had made its appearance leucocytes were injected. Three similar injections were subsequently given and at the end of 27 days dullness over the right chest was only slightly greater than that before inoculation; the changes are shown by Chart 1. During this period the body weight diminished slightly. The animal at the end of eight months is very active and apparently well, its weight being 2,050 grm. more than the weight at the time of inoculation. Percussion shows only a normal relative dullness over the right thorax.

Control Experiments.

EXPERIMENT 7.—Dog, wt. 5,050 grm. Control. The weight of the animal fell quickly after inoculation. Relative dullness over the right pleural cavity gradually increased and absolute dullness was present at the end of a week. Absolute dullness disappeared although relative dullness persisted until a short time before death. Death occurred after onset of cough and purulent nasal discharge at the end of 34 days.

Day of Diseas.	Absolute Dullness.	Relative Dullness.
1	—	3.1
8	4.5	6.0
11	4.5	6.0
15	—	4.5
21	—	4.5
25	—	6.1
28	—	6.1
35		

Inoculated with *B. tuberculosis*.

Died.

Autopsy.—The animal is emaciated. In the right chest wall at the site of inoculation is a mass of partially caseous tuberculous tissue which projects upon the parietal pleura. Each pleural cavity contains only about 25 c.c. of fluid. The thickened and injected mediastinum and adjacent membranes contain tuberculous masses. The substernal, retropleural and retroperitoneal lymphatic glands are much enlarged and tuberculous. There is bronchitis and patches of broncho-pneumonia. The liver is enlarged, and exhibits fatty degeneration; small caseous tubercles are numerous.

EXPERIMENT 8.—Dog, wt. 7,250 grm. Control. After inoculation the body weight rapidly fell; relative dullness over the right pleural cavity increased slightly and at the end of a week absolute dullness had made its appearance but subsequently disappeared. Death occurred at the end of 35 days.

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Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	5.6	Inoculated with <i>B. tuberculosis</i> .
9	3.3	6.1	
12	3.3	6.1	
16	3.3	5.2	
20	3.3	5.2	
22	—	6.1	
29	—	6.1	Died.
36			

Autopsy.—The animal is emaciated. Each pleural cavity contains about 150 c.c. of turbid yellowish fluid. The mediastinum and subpericardial membranes which are intensely injected contain caseous masses. The substernal lymphatic glands and lymphatic glands near the duodenum are enlarged and caseous. The lungs contain deep red patches of broncho-pneumonia and there is bronchitis. The liver contains numerous small caseous tubercles.

The first injection of leucocytes into a pleural cavity containing effusion, indicated by absolute and increased relative dullness, has in two instances (Experiments 5 and 6) been followed within twenty-four hours by diminution of dullness, doubtless the result of absorption of fluid. In one instance (Experiment 4) fall of the level of dullness was delayed at least twenty-four hours whereas in one instance (Experiment 3) a well-marked increase of dullness preceded the disappearance of absolute dullness, relative dullness, probably due to the presence of tuberculous masses, persisting. Injections of leucocytes were repeated at intervals of a week or ten days, dullness not infrequently increasing between injections and falling after them. These changes are illustrated by Chart I, in which relative and absolute thoracic dullness are charted.

In this series of experiments, which were begun eight months ago, two control animals died at the end of five weeks; two animals receiving three injections of leucocytes lived about two months; a third injected animal, receiving four injections, lived three months, and a fourth animal receiving the same number of injections is living and well.

It is noteworthy that injection of a large quantity of leucocytes (20 c.c.) has been usually followed by marked increase of dullness with subsequent fall. The following experiments show the effect of leucocytic injections repeated more frequently and in larger quantity than those previously employed.

Series B.—The effect of leucocytes injected into the pleural cavity at short intervals and in large quantity during the course of tuberculous pleurisy.

Two animals were inoculated intrapleurally with 0.5 cubic centimeter of a suspension of *Bacillus tuberculosis*. During the first ten days changes of thoracic dullness pursued an approximately parallel course in the two animals, relative dullness gradually increasing and absolute dullness appearing. The injected animal died at the end of fifty-six days and the control animal was immediately killed for comparison.

EXPERIMENT 9.—Dog, wt. 5,800 grm. At the end of a week a nodular thickening had developed in the chest wall at the point of inoculation; leucocytes were injected into the nodule which after several weeks diminished in size. Leucocytes were injected into the pleural cavity ten days after inoculation and the animal received four injections within fifteen days. Mange made its appearance and was widely distributed upon the skin. The animal became thin and death occurred at the end of 56 days.

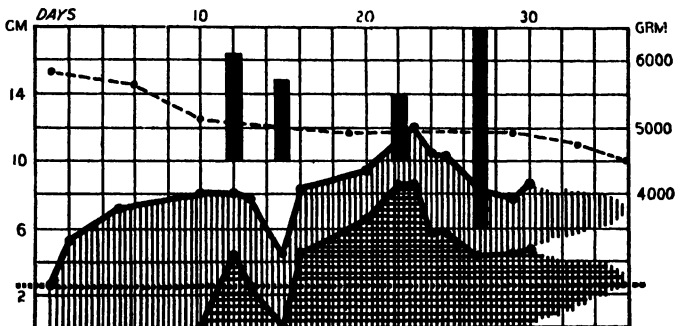


CHART 2. Experiment 9; injection of leucocytes.

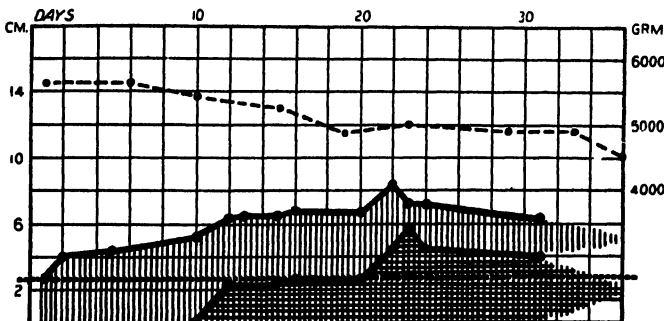


CHART 3. Experiment 10; control.

Autopsy.—The body is thin but not emaciated. The right pleural cavity is distended with several hundred cubic centimeters of almost opaque whitish fluid which compresses the lung. The parietal and pulmonary pleura is everywhere grayish white and thickened often to 1 mm. The left pleural cavity is also distended and the pleura is grayish white but less thickened than on the right side. The distribution of the lesions which are present is represented by Fig. 3; compare with Fig. 2 showing the lesions in the control. Upon the surface

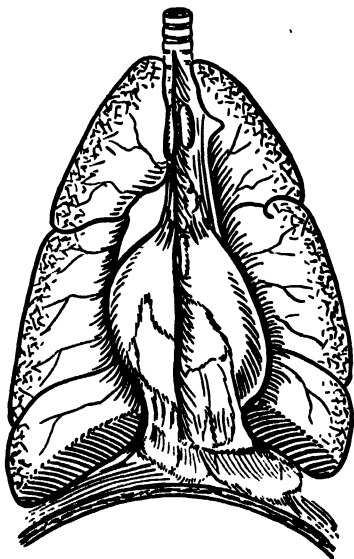


FIG. 3. Diagram of lesion of Experiment 9; compare with control represented by Fig. 1.

Autopsy.—The right pleural cavity contains about 100 c.c. of turbid fluid; the left cavity contains about the same amount of fluid. The parietal and pulmonary pleurae are not thickened. Situated in the mediastinum above the diaphragm and extending into the subpericardial membrane is a very large mass of grayish white succulent, in places, caseous tissue (see Figs. 1 and 2); a similar mass which is smaller occupies the subpericardial membrane on the right side. Figs. 1 and 2 show the situation of these tuberculous masses. A large mass of tuberculous tissue is situated in the posterior mediastinum above the diaphragm. The substernal lymphatic glands are greatly enlarged, hard and caseous, measuring 1.6 cm. in long diameter. The lungs contain no tubercles. The liver contains small scattered tubercles.

Whereas increase of thoracic dullness proceeded uninterruptedly in the control, the first intrapleural injection of leucocytes in the treated animal was followed by a fall of relative and disappearance of absolute dullness. A second injection three days after the first

of the mediastinum which is thickened and leathery are several flat yellowish white elevations. At the junction of the subpericardial membranes and diaphragm on each side are small scar-like masses which on section are composed of grayish white tissue. The substernal lymphatic glands are moderately enlarged, measuring 1.4 cm. in long diameter. The lungs, which are much compressed, contain numerous tubercles. The liver contains a great number of large tubercles; tuberculous tissue occupies at least a third of a section for microscopic examination.

Control Experiment.

EXPERIMENT 10.—Dog, wt. 5,650 grm. Control. Ten days after inoculation a nodule made its appearance immediately below the skin at the site of injection; it gradually increased in size and broke two weeks later. There was cough beginning about ten days after inoculation. Weight diminished gradually. The animal was killed at the end of 56 days, for comparison with that of the preceding experiment.

was followed by an accumulation of fluid which showed no tendency to subside until a third injection was given. The fourth injection (30 c.c) was given with the hope of influencing favorably by a large quantity of leucocytes what appeared from the extent of thoracic dullness to be a very severe infection. Fluid showed little indication of decrease and death resulted about one month later. The generalized chronic pleurisy with effusion, which doubtless caused or hastened death, has not been observed in any of the untreated tuberculous animals and is probably referable to the injections of leucocytes which were repeated at unusually short intervals and in unusual amount; for repeated observations have demonstrated that the intensity of the inflammatory reaction which follows injection of leucocytes bears a relation to the quantity injected.

Although the injection of leucocytes did not prolong the life of the animal nor exert a favorable influence upon the course of the disease, comparison of the lesion with that of a control animal inoculated with the same suspension and killed after the same interval has shown that the local tuberculous process has been markedly retarded. In the control animal (Fig. 1) the mediastinum and sub-pericardial membranes are occupied by enormous succulent partially caseous masses and the lymphatic glands adjacent to the pleural cavities are greatly enlarged and caseous. In the treated animal (Fig. 3) there are small scar-like masses almost wholly composed of fibrous tissue in the same situations and the adjacent lymphatic glands are moderately enlarged and show no caseation. Microscopic examination of the thickened pleura shows that it is composed of fibrous tissue with none of the characters of tuberculous new growth. Tuberculosis in the neighborhood of the injected pleural cavity had in large part disappeared.

Series C.—The effect of long-continued injections of leucocytes upon the course of experimental tuberculous pleurisy.

With the information derived from the foregoing experiments, an attempt was made to treat with leucocytic injections animals with experimental tuberculous pleurisy. Seven dogs received half a cubic centimeter of the same suspension of tubercle bacilli and at the end of ten days there was in all of them a well-marked increase of thoracic dullness. Three animals were kept as controls whereas the remaining four received repeated injections of leucocytes.

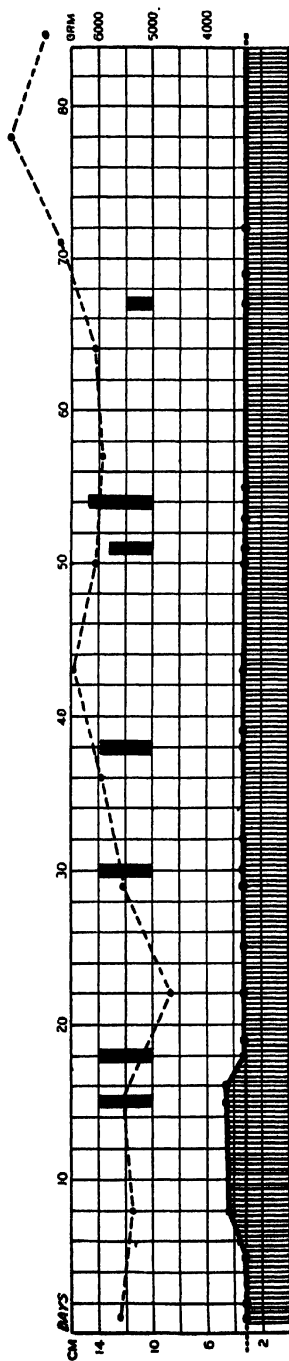


CHART 4. Experiment 11; injection of leucocytes.

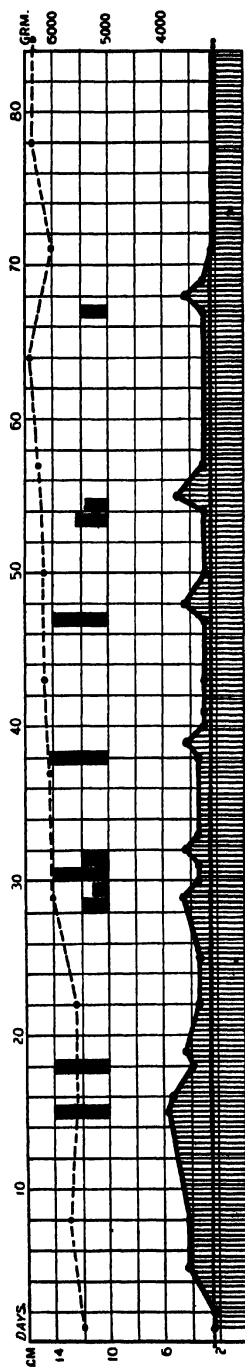


CHART 5. Experiment 12; injection of leucocytes.

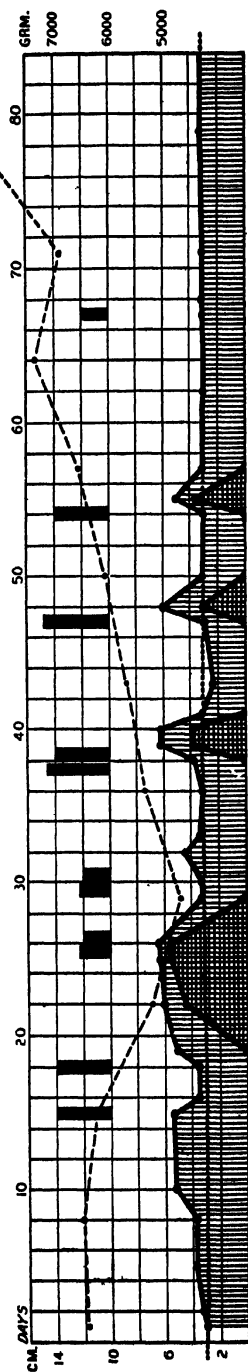


CHART 6. Experiment 13; injection of leucocytes.

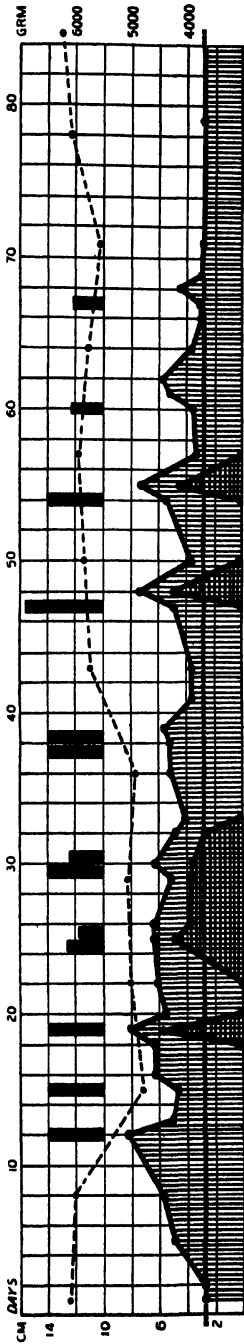


CHART 7. Experiment 14; injection of leucocytes.

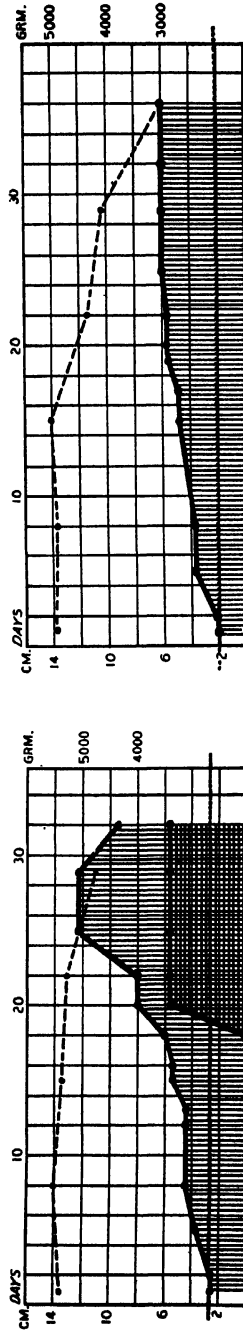


CHART 8. Experiment 15; control.

CHART 9. Experiment 16; control.

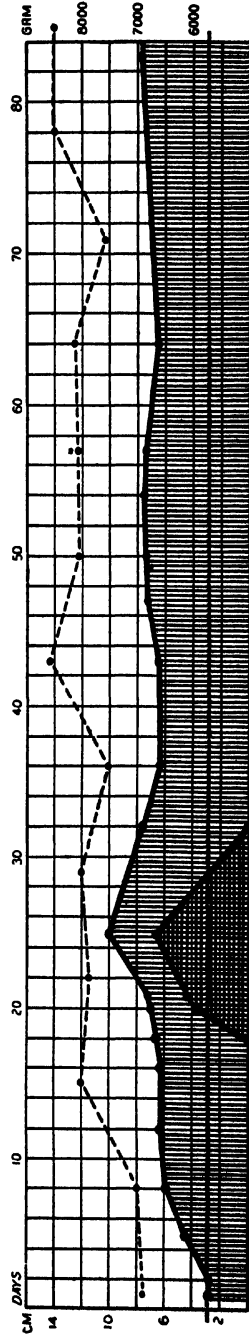


CHART 10. Experiment 17; control.

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EXPERIMENT 11.—Dog, wt. 5,650 grm. After inoculation the animal lost about 1,000 grm., the most marked loss in weight following the first two injections of leucocytes. After four weeks the animal regained and subsequently much exceeded its original weight. Thoracic dullness which had increased as the result of inoculation disappeared after the first leucocytic injection and did not reappear (Chart 4). The animal received into the right pleural cavity seven injections. On the twenty-fifth day of the disease a nodule appeared upon the chest wall caused doubtless by infection of the needle tract at the time of inoculation. Leucocytes were twice injected into the nodule which at one time measured 4 cm. across; it diminished in size and almost completely disappeared two weeks after its appearance. The nodule reappeared at the same site and after two weeks was 2 cm. across; four injections of leucocytes (1 c.c.) were not followed by any diminution of size and after attaining a diameter of 4 cm. the nodule broke and discharged during several weeks upon the surface. Healing with scar formation followed. The animal increased much in weight and became strong and active. It acquired the habit of bounding to the top of its cage and would jump continuously during several hours. Five months after inoculation it suddenly became sick and died two days later. At the time of death it was well nourished, weighing one thousand grams more than at the time of inoculation.

Autopsy.—Fat in the subcutaneous tissue and elsewhere is abundant. The pleural cavities contain no fluid. The mediastinum is delicate and membranous and very redundant so that it can be pouched far to the right or left; the membranes below the pericardium are delicate and exhibit the same redundancy. In these membranes and upon the surface of the diaphragm are small, little elevated, patches with reddish gray color; there is no tuberculous tissue and the pleural surfaces are smooth and glossy. The mediastinal lymphatic glands are slightly enlarged, measuring 1 cm. in length and are red and succulent.

The liver is apparently normal. The duodenum near the stomach for a distance of about 7 cm. is plum-colored and apparently in part gangrenous; the adjacent mesentery including the entire pancreas with the exception of a small part of the duodenal arm is infiltrated with blood; the adjacent lymphatic glands particularly those near the liver are enlarged and hemorrhagic. There is no fat-necrosis.

EXPERIMENT 12.—Dog, wt. 5,530 grm. Following inoculation there was slight gradual increase of weight. Thoracic dullness steadily increased during two weeks and diminished immediately after the first injection of leucocytes. During the eleven days between the second and third injection there was increase of dullness which again fell to a level little above that before inoculation. Subsequent elevations above this level were in every instance the result of injection into one or both pleural cavities. In Chart 5 and in subsequent charts injection into both pleural cavities is indicated by two heavy lines side by side, the length of these lines representing the quantity of leucocytes employed. One week after inoculation a nodule appeared in the chest wall at the site of puncture; during ten weeks the nodule received ten injections of leucocytes (0.5 to 1 c.c.). During this time it increased to a maximum diameter of 4 cm. and gradually disappeared without discharging upon the surface. After the fourth intrapleural injection there was cough; an area of relative dullness 5 cm. across

with a peculiar hard resistant character on percussion appeared about the site of injection and persisted several days; cough disappeared. The animal five months after inoculation is well and weighs 1,120 grams more than before inoculation.

EXPERIMENT 13.—Dog, wt. 6,450 gm. After inoculation thoracic dullness gradually increased but its level rapidly fell immediately after the first injection of leucocytes (Chart 6); it rose to a high level after the second injection, and absolute dullness appeared but it fell to normal after the third injection which was made into both pleural cavities. At this time the animal was very sick and there was cough and purulent discharge from the nose and eyes; body weight had diminished 1,700 gm. and the animal was very thin. After the fourth week weight steadily increased and evidences of bronchitis disappeared. After the effect of the fourth injection had disappeared there was (on the thirty-eighth day) an increase of thoracic dullness but subsequent elevations (see chart) occurred only as the immediate result of leucocytic injections.

Three weeks after inoculation induration appeared along the tract marked by the inoculating needle; this nodule received one injection of leucocytes (0.5 c.c.) and lying immediately below the skin broke upon the surface. Two subsequent injections were made but it persisted until the seventeenth week after inoculation, disappearing finally.

The animal is large and strong and having grown considerably, its weight is 4,500 grm. greater than before inoculation.

EXPERIMENT 14.—Dog, wt. 6,150 grm. After inoculation thoracic dullness rose quickly to a high level (Chart 7), but fell considerably immediately after the first injection of leucocytes. Body weight diminished rapidly and the animal became very thin. After the second leucocytic injection the level of dullness increased and absolute dullness appeared. Changes following the six subsequent injections, of which with three there was injection into both the right and left pleural cavities, were almost constant, namely, fall of the level of dullness, either immediately or after a preliminary increase, to a level below that at the time of injection and subsequently, after the effect of the injection had disappeared, an increase of dullness. Between succeeding injections the level of dullness became gradually lower and after the immediate effect of the ninth injection had subsided there was no elevation, subsequent increase of dullness occurring only as a sequence of leucocytic injection. After the fifth week of the disease the animal began to gain weight.

A nodule which developed after two weeks at the site of inoculation attained a diameter of 2 cm.; after injection of 0.5 c.c. of leucocytes there was no increase of size. A second injection was made. The nodule diminished in size and during the sixth week of the disease was represented only by induration at its former site; a third injection was made in the neighborhood of the indurated tissue.

After the second intrapleural injection of leucocytes extensive emphysema of the subcutaneous tissue on the right side of the body made its appearance and disappeared after four days.

The animal is well and strong and weighs 400 grm. more than at the time of inoculation.

Control Experiments.

EXPERIMENT 15.—Dog, wt. 5,300 grm. Control. Thoracic dullness increased gradually during the first three weeks after inoculation and subsequently more rapidly. The animal became thin and died at the end of 35 days. The fall of dullness (and small amount of effusion found at autopsy) suggest that there was rapid absorption of fluid just before death (Chart 8).

Autopsy.—The right pleural cavity contains 25 c.c. of reddish serous fluid; the left cavity contains 5 c.c. Throughout the mediastinum are masses of hard caseous tissue, the largest being situated just above the diaphragm; similar masses occur in the subpericardial membranes on either side. Flat, gray white nodules occur upon the posterior surface of the right lung. The middle lobe of the right lung exhibits pneumonic consolidation. The substernal lymphatic glands are greatly enlarged and firmly caseous; enlarged hard glands are found near the pancreas and in the retroperitoneal tissue beside the adrenal glands.

EXPERIMENT 16.—Dog, wt. 4,950 grm. Control. Thoracic dullness gradually increased from the time of inoculation until death (Chart 9). At the end of about three weeks the animal was very thin and there was abundant purulent discharge from the nose and eyes; an ulcer formed upon the right cornea. Death occurred at the end of 36 days.

Autopsy.—The pleural cavities contain no fluid; the mediastinum is injected and contains small nodules. Above the diaphragm is a caseous mass about 1 cm. across. The substernal lymphatic glands are moderately enlarged and caseous. At the bifurcation of the trachea is a mass of caseous lymphatic glands which encircle and compress the right bronchus. The lungs contain numerous patches of pneumonic consolidation.

EXPERIMENT 17.—Dog, wt. 6,800 grm. Control. After inoculation of the animal, a stout pug, weight rapidly increased and continued much greater than before inoculation. Nevertheless relative dullness increased steadily and absolute dullness made its appearance, disappearing later (Chart 10). Almost immediately after inoculation a nodule appeared at the site of puncture. The nodule increased greatly in size, broke through the skin and discharged during several weeks. The mass below the skin diminished in size and finally disappeared, leaving a small scar. Absolute dullness disappeared at the end of five weeks, but abnormal relative dullness has persisted until the present time. The animal (at the end of five months) is very fat and apparently well, weighing 1,450 grm. more than before inoculation.

Of the animals which were inoculated as controls two died at the end of five weeks, a time corresponding to the time of death of the controls of Series A. The third animal used as control, a relatively large dog, was little affected by the pleural and subcutaneous tuberculosis with which it was infected, but, on the contrary, increased considerably in weight; nevertheless increased thoracic dullness did not return to normal.

Of four tuberculous animals injected with leucocytes one (Experiment 11) exhibited normal thoracic dullness after the third

week and a second (Experiment 12) after the sixth week. In two animals treated with leucocytes the disease was much more severe and there was great loss of body weight. One animal (Experiment 13) passed through a stage in which there were physical signs of fluid in considerable amount, and exhibited normal thoracic dullness only after the sixth week. In the remaining animal (Experiment 14) there was, after the effect of each injection had subsided, an increase of dullness, doubtless referable to the tuberculous process which was still active. Each injection during this period after a primary rise depressed thoracic dullness to a somewhat lower level so that after the seventh week there was no increase except as the result of injection of leucocytes.

In this series of experiments leucocytic injections were at first made at short intervals in relatively small quantity—approximately ten cubic centimeters. Even after thoracic dullness had returned to a level approaching that before inoculation with tuberculosis, leucocytic injections were continued. Since numerous examinations had shown that the tuberculous lesion was bilateral, injections were often made simultaneously into the two cavities.

Series D.—The effect upon thoracic dullness of tubercle bacilli and leucocytes injected simultaneously; the effect of leucocytes preserved during several days at low temperature.

Since the previous experiments have afforded evidence that leucocytes injected into the plural cavity already infected with tuberculosis retard the development of the lesion and tend to cause its disappearance, the possibility has suggested itself that injection of leucocytes, together with tubercle bacilli, might prevent the onset of tuberculosis. The clinical course of the disease in two animals immediately after injection of a mixture of ten cubic centimeters of leucocytes with half of a cubic centimeter of a suspension of tubercle bacilli gave some support to belief that the organism had been wholly destroyed; nevertheless at the end of two weeks such well-marked increase of thoracic dullness occurred that there was no doubt that tuberculosis had developed. The animals were subsequently used to test the efficiency of cells which had been preserved from twenty-four to forty-eight hours at a low temperature, several degrees above the freezing point. One of the animals which

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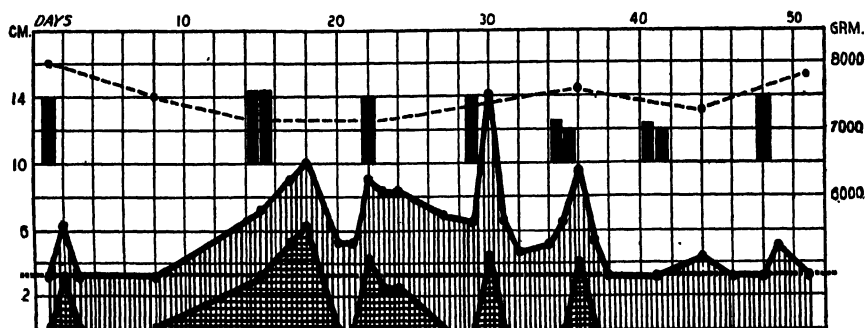


CHART 11. Experiment 18; inoculation with *B. tuberculosis* and leucocytes; injection of leucocytes.

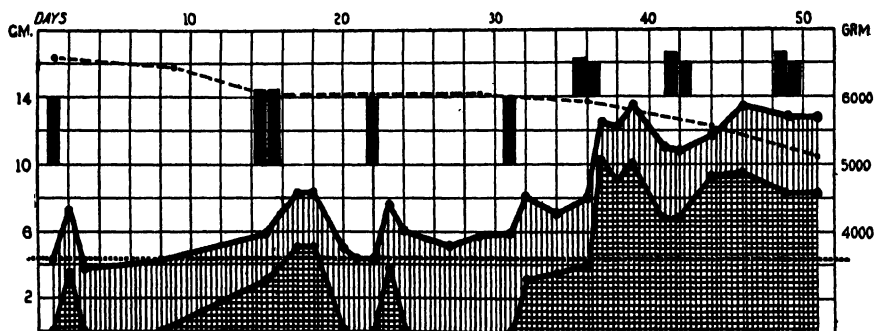


CHART 12. Experiment 19; inoculation with *B. tuberculosis* and leucocytes; injection of leucocytes preserved at low temperature.

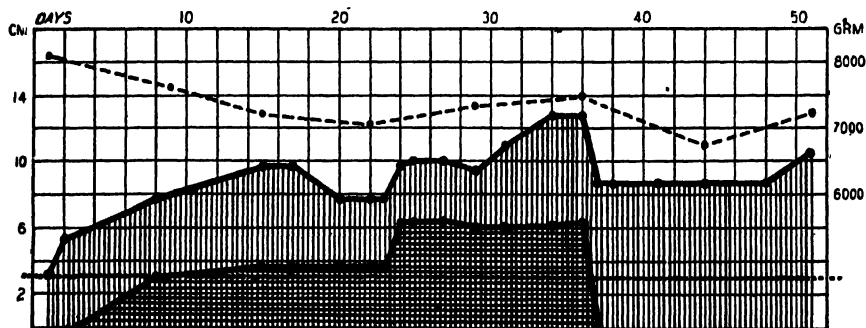


CHART 13. Experiment 20; control.

had received tubercle bacilli and leucocytes was subsequently injected with leucocytes freshly obtained, whereas the second animal which had received the same mixture was injected with leucocytes which had been preserved in cold storage. In some instances the cells employed for corresponding injections in the two animals were identical, the animal in Experiment 19 receiving its injection one or two days after that of Experiment 18. Experiment 20, in which tubercle bacilli alone had been employed, served as control.

EXPERIMENT 18.—Dog, wt. 7,950 grm. The clinical course after inoculation with a mixture of tubercle bacilli and leucocytes is shown in Chart 11. The animal was given after the inoculating injection six injections of freshly obtained leucocytes and was killed at the end of 50 days; it was strong and active when killed.

Autopsy.—The pleural cavities contain no fluid. Several flat fibroid patches occur upon the surface of the lungs, but the pleural surfaces are almost free from evidence of tuberculosis. The subpericardial membranes and mediastinum in front of the heart are delicate save for the presence of a thin firm mass of tuberculous tissue about 7 mm. across. The mediastinum above the level of the heart is thickened and contains several indurated nodules. The substernal lymphatic glands are soft, moderately enlarged, and contain several caseous foci. In the lungs are miliary tubercles. The liver contains numerous small tubercles of which many have a delicate fibrous capsule. In the kidney are a few opaque tubercles.

EXPERIMENT 19.—Dog, wt. 6,700 grm. The clinical course after inoculation, identical with that of Experiment 18, is shown in Chart 12. A nodule appeared at the site of inoculation and after receiving four injections (0.5 to 1 c.c.) of leucocytes kept at low temperature almost completely disappeared, but although injected three times subsequently increased to a diameter of nearly 4 cm. The animal received into the pleural cavities six injections of leucocytes which had been preserved in cold storage during one or two days. The animal which was very weak was killed after 50 days for comparison with the preceding.

Autopsy.—The subcutaneous tissue is jaundiced. The pleural cavities each contain 200 c.c. of fluid. Upon the pleural surfaces are elevated plaques of tuberculous tissue. The mediastinum from the lymphatic glands at the base of the neck, which are enlarged to a diameter of 3 cm., to diaphragm is converted into a thick crinkled mass by partly caseous tissue. Similar masses of large size occupy the subpericardial membranes and are scattered over the right parietal pleura and surface of the diaphragm. In the substance of the right lung corresponding to the site of inoculation is a tuberculous mass; the bronchial lymphatic glands on the same side are much enlarged and tuberculous. The lungs contain tubercles. The liver is enlarged and beset with numerous large tubercles, occupying on section at least a third of the tissue.

Control Experiment.

EXPERIMENT 20.—Dog, wt. 8,050 gm. Control. The animal received 0.5 c.c. of the suspension of tubercle bacilli employed in the two preceding experiments mixed with 10 c.c. of 0.85 per cent. sodium chloride solution. The clinical course is shown in Chart 13. The animal was killed after 50 days.

Autopsy.—The right pleural cavity contains 2 c.c. of red turbid fluid; the left cavity contains 15 c.c. Upon the surfaces of the lungs and upon the diaphragm are numerous flat tubercles. The mediastinum and subpericardial membranes are thickened and beset with numerous tubercles. A hard mass of gray white tissue (1.2 cm. across) is situated in the right, a second (2 cm. across) in the left subpericardial membrane at its junction with the diaphragm; a third mass is situated in the mediastinum above the diaphragm. The substernal lymphatic glands are moderately enlarged, hard and almost wholly caseous. In the lungs are miliary tubercles. The liver contains numerous tubercles; in the kidney opaque tubercles often 1.5 cm. in diameter are fairly numerous.

In the animal of Experiment 20, used as control, relative and absolute thoracic dullness increased gradually after inoculation, but diminished suddenly after the thirty-fifth day; nevertheless relative dullness maintained a high level until the animal was killed. In the dog of Experiment 18, which received fresh leucocytes, the clinical course was identical with that illustrated by Experiments 11 to 14 of Series C, namely, depression of dullness, perhaps preceded by temporary increase, after each injection. Comparison of these two animals of the present series confirms the result of former experiments and affords clinical and anatomical evidence that the presence of artificially introduced leucocytes has retarded the development of the tuberculous lesion.

The employment of leucocytes which have been preserved at a low temperature has not had an equally favorable result, but the experiment is indecisive, for autopsy has shown that the lung has been punctured at the time of inoculation. Tuberculosis of the lung and of the bronchial glands doubtless explains in part the rapid progress of the disease. Nevertheless the charted thoracic dullness shows that changes which follow injection of leucocytes kept at low temperature during several days may be identical with those caused by freshly obtained leucocytes. Injection on the fifteenth day of the disease was followed after an interval of several days by diminution of relative dullness and disappearance of absolute dullness referable to diminution of the fluid contents of the chest.

Series E.—The clinical and pathological changes following simultaneous injection of tubercle bacilli and leucocytes.—Injection of leucocytes together with tubercle bacilli in the preceding experiments has been followed by the reaction which occurs when leucocytes are injected into the normal pleural cavity (see Charts 11 and 12); there is accumulation of fluid which quickly disappears. Subsequent increase of dullness, which is the otherwise constant result of tuberculous pleurisy, is delayed. The same experiment has been repeated and, in order that the resulting changes may be compared by anatomical examination, the animals have been killed as soon as that which has received leucocytes has exhibited increase of thoracic dullness.

EXPERIMENT 21.—Dog, wt. 5,950 grm. A suspension of tubercle bacilli (0.5 c.c.) mixed with 10 c.c. of leucocytes which had been kept at a temperature slightly above freezing during three days was injected into the right pleural cavity. The level of thoracic dullness (Chart 14) rose abruptly, subsided, and remained normal during at least a week; it then rose and the animal was killed 16 days after inoculation.

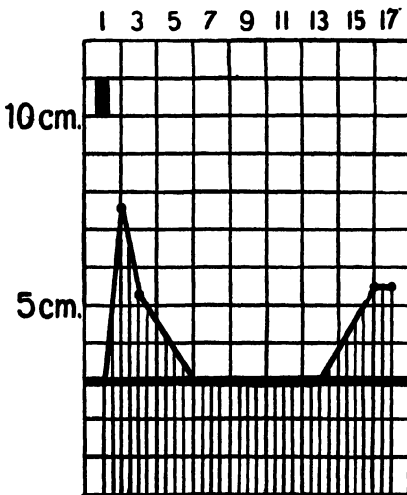


CHART 14. Experiment 21; inoculation with *B. tuberculosis* + leucocytes.

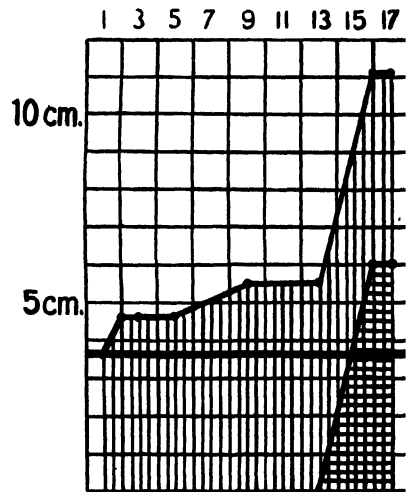


CHART 15. Experiment 22; control.

Autopsy.—The right pleural cavity contains a small amount of turbid whitish fluid (not measured); the left cavity contains a somewhat greater quantity. The mediastinum is delicate and contains a few tubercles; the right and left subperi-

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cardial membranes are studded with tubercles. Just above the diaphragm is a small, firm, succulent mass. The mediastinal lymphatic glands are moderately enlarged, measuring 1.2 cm. The surfaces of the diaphragm and the parietal pleuræ are smooth. The liver contains small miliary tubercles.

EXPERIMENT 22.—Dog, wt. 6,900 grm. Control. A suspension of tubercle bacilli (0.5 c.c.) was diluted with physiological salt solution to 10 c.c. and injected into the right pleural cavity. Thoracic dullness (Chart 15) increased gradually during 12 days and then more rapidly. The animal was killed at the end of 16 days.

Autopsy.—The right pleural cavity contains 120 c.c. turbid, whitish, coagulable fluid; the left cavity, 95 c.c. Upon the surface of the diaphragm and upon the parietal pleura in greatest abundance on the right side are large, flat, gray white nodules. The mediastinum is thick and beset with small tubercles and partially caseous masses, the largest being just above the diaphragm; the subpericardial membranes on either side contain large hard masses of gray white tissue. The substernal lymphatic glands are considerably enlarged, measuring 1.7 cm. The liver contains small miliary tubercles.

The changes of thoracic dullness observed in Experiment 21 after inoculation with tubercle bacilli mixed with leucocytes are identical with those noted immediately after injection of the same mixture in two animals of the preceding series; in each series there has been the same contrast with the control. The persistence of normal thoracic dullness during a week after the preliminary rise and fall suggests that the development of the tuberculous process has been retarded. Twenty-four hours after the appearance of abnormal dullness in the animal which received leucocytes both animals of the present series were killed; effusion was abundant in the control but much less in the animal with leucocytes; tuberculous pleural nodules were numerous and massive in the former, but almost absent in the latter; the membranes within the thorax contained abundant tuberculous tissue in the one, but little in the other. The substernal lymphatic glands were much larger in the control.

The experiment furnishes additional evidence that leucocytes tend to check the development of a tuberculous lesion, even though they have been preserved during several days at a temperature a little above the freezing point.

Series F.—*Anatomical changes found in animals with tuberculous pleurisy killed after repeated injections of leucocytes.*

In the following series of experiments both control and animal treated by intrapleural injections of leucocytes were killed at the

same interval after inoculation in order to determine by anatomical examination the effect of the injected cells. The animals in the two following experiments were of unequal size, that which received injections being a small puppy; each received into the right pleural cavity half of a cubic centimeter of the same suspension of tubercle

EXPERIMENT 23.—Dog, wt. 4,150 gm. During the first two weeks after inoculation relative thoracic dullness over the infected cavity increased from 3 to 7.7 cm. Injection of leucocytes (16 c.c.) on the fourteenth day was followed by a fall of this level. Two subsequent injections caused diminution of the area of dullness which after the third week twice returned to normal but rose slightly after the effect of the injection had disappeared. Twenty-four hours before the animal was killed relative dullness measured 4.4 cm. and the animal received into the right pleura 9.5 c.c. leucocytes. Cough and purulent nasal discharge appeared during the third week of the disease and persisted until death. There was a nodule at the site of inoculation which received one injection of leucocytes; it did not increase in size but remained until death. The animal was killed after 32 days.

Autopsy.—The right pleural cavity contains 12 c.c. turbid blood-stained fluid; the left, 11 c.c. The parietal pleura is smooth save at one point corresponding to the site of inoculation where there is a group of small nodules; upon the opposing surface of the lung and extending into the substance in an area of fibrous tuberculous tissue. The bronchial lymphatic glands on the right side are enlarged and tuberculous. In the mediastinum and subpericardial membranes are tuberculous masses of considerable size. The substernal lymphatic glands are greatly enlarged and caseous. Tuberculous glands are found near the pancreas. The lungs contain patches of broncho-pneumonia and miliary tubercles.

EXPERIMENT 24.—Dog, wt. 7,000 gm. Control. Two weeks after inoculation relative dullness had increased from 3 to 8.6 cm. and absolute dullness had made its appearance; on the eighteenth day relative dullness was 12 cm. and absolute dullness 8.5 cm. These levels were maintained until the animal was killed 32 days after inoculation. A nodule appeared at the site of inoculation and broke upon the surface of the skin.

Autopsy.—The right pleural cavity contains 235 c.c., the left 184 c.c. of turbid pale yellow fluid. The pleural surfaces are intensely injected and the parietal pleura is thickly beset with flat tuberculous nodules which near the diaphragm are confluent. The mediastinum is occupied by a large mass of hard tuberculous tissue extending into the left subpericardial membrane. The substernal lymphatic glands are greatly enlarged and caseous; tuberculous glands occur near the pancreas. The lungs contain miliary tubercles.

Injection of leucocytes was followed by diminution of thoracic dullness already increased by tuberculous pleurisy but even before the animal was killed it was evident that the tuberculous process was still active, for slight increase of dullness had occurred just before death. Difference between injected and uninjected animals

was well marked in the pleural cavities; in the control there was fluid in great quantity, the pleural membranes were injected and raised tuberculous plaques were present in immense numbers upon the parietal pleura, whereas in the injected animal the pleural surfaces were smooth and the cavities contained very little fluid. Elsewhere, both in the control and in the injected animal, tuberculosis was advanced and widely disseminated. It is noteworthy that the lung had been punctured at the time of inoculation, so that, although as the subsequent course of the disease showed, the pleura had been infected, there was tuberculosis of the lung and of the bronchial lymphatic glands as well.

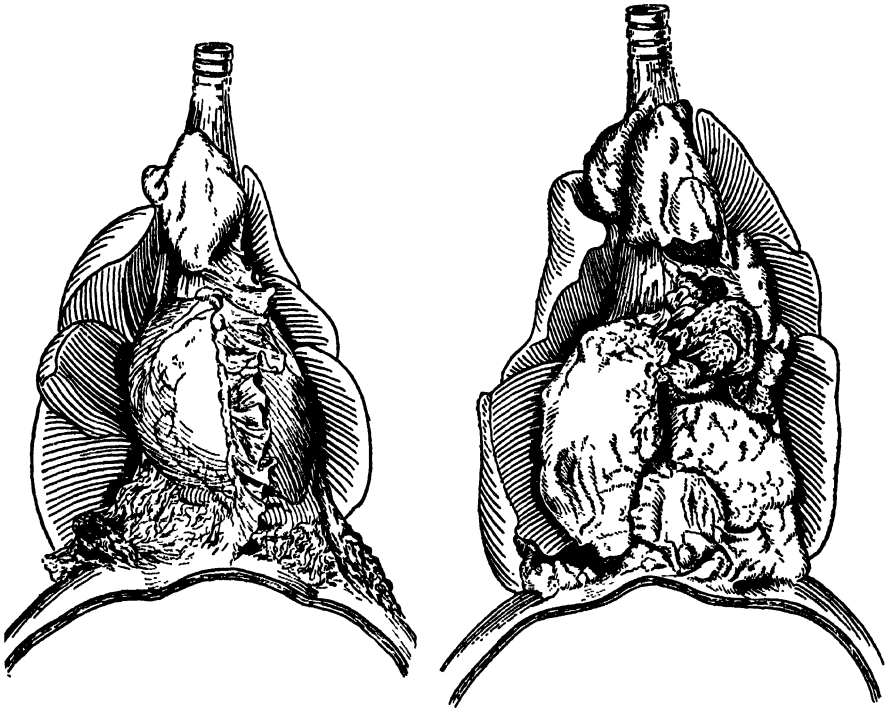
The following experiments are described to illustrate further the anatomical effect of injected leucocytes. They confirm those which have already been cited and furnish additional evidence that injected leucocytes influence in greatest degree tuberculosis in structures with which they come into immediate contact. One animal, used as control, received a suspension of tubercle bacilli (0.5 c.c.). The other animal received the same suspension (0.5 c.c.) mixed with leucocytes (10 c.c.); subsequently leucocytes were injected into one or both pleural cavities at intervals of about a week.

EXPERIMENT 25.—Puppy, wt. 5,650 grm. After simultaneous injection of leucocytes and tubercle bacilli there was no permanent increase of thoracic dullness until the sixth day when it had increased from 3.2 to 3.7 cm. Subsequent injections of leucocytes into one or both pleural cavities reduced this level and it remained at the normal level save as the immediate result of injection until death. There was bronchitis and the animal lost weight. The animal was killed after 33 days.

Autopsy.—The pleural cavities contain no fluid and there is no tuberculous tissue in the adjacent membranes. The mediastinum and subpericardial membranes are delicate and evidently greatly stretched so that they form redundant folds and can be pouched far to the right or left (Fig. 4). The parietal and visceral pleurae are smooth except for patches and shreds of soft reddish tissue which represent perhaps the site of tuberculous plaques. The substernal lymphatic glands are enormously enlarged, measuring 3.5 cm. in length; they are homogeneously caseous and surrounded by a fibrous capsule. The lungs contain an occasional miliary tubercle; the liver contains innumerable tubercles.

EXPERIMENT 26.—Puppy, wt. 6,050 grm. Control. Thoracic dullness, measuring before inoculation 2.8 cm., increased continuously after inoculation; there was cough and purulent discharge from the nose after the third week. A tuberculous nodule formed at the site of inoculation. At the end of 32 days relative dullness had increased to 8.2 cm., absolute dullness to 4.6 cm. The animal was killed at the end of 33 days (24 hours before death 10 c.c. of leucocytes had been injected into the right pleural cavity).

Autopsy.—The right pleural cavity contains 170 c.c. of turbid fluid; the left, 125 c.c. of less turbid coagulable fluid. The parietal pleura is injected and rows of tuberculous plaques are situated between the ribs; similar plaques occur upon the diaphragm. The mediastinum above the diaphragm contains a tuberculous mass of great size extending into both subpericardial membranes which are thickened and studded with tubercles (Fig. 5). The mediastinum above this mass is thickened and beset with tubercles and larger tuberculous masses. The substernal lymphatic glands are enormously enlarged, 3.5 cm. in length and caseous. Tubercles are moderately numerous in the lungs and are present in enormous number in the liver.



4. Experiment 25; animal injected with leucocytes.

FIG. 5. Experiment 26; control.

In the animal which had received leucocytes the pleural membranes exhibited no evidence of tuberculosis whereas in the control there was pleural effusion and advanced tuberculosis of the pleura and mediastinum (compare Figs. 4 and 5). Nevertheless tubercle bacilli had found lodgment in the substernal lymphatic glands of the injected animal; tubercle bacilli perhaps disseminated from this focus had caused miliary tuberculosis of lungs and liver.

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In the following experiment repeated injection of leucocytes had little effect upon the development of tuberculosis.

EXPERIMENT 27.—Dog, wt. 4,850 grm. On the fifth day after simultaneous injection of tubercle bacilli (0.5 c.c.) and leucocytes thoracic dullness had increased to 4.3 cm., the original level being 2.6 cm. Injection of leucocytes repeatedly reduced thoracic dullness but failed to restore it to normal. Cough appeared and was accompanied by purulent nasal discharge, conjunctivitis and ulceration of the right cornea. The animal lost weight and became sick and weak. It was killed after 39 days; just before death relative dullness measured 5.5 cm., absolute dullness 2.8 cm.

Autopsy.—The right pleural cavity contains 5.5 c.c. of almost clear fluid; the left cavity contains 10 c.c. of very turbid yellowish fluid. Upon the parietal pleura and diaphragm is an occasional tuberculous plaque. The mediastinum from substernal glands which are greatly enlarged and caseous to diaphragm is occupied by yellowish white tissue which is partly caseous and partly fibrous. Almost the entire right lung is consolidated by mottled red and gray patches of broncho-pneumonia; the left lung contains similar patches. The liver contains tubercles; lymphatic glands near the pylorus are enlarged and caseous.

EXPERIMENT 28.—Dog, wt. 4,400 grm. Control. Thoracic dullness (normal relative dullness measured 3.2 cm.) increased continuously after inoculation with 0.5 c.c. suspension of *B. tuberculosis* mixed with 10 c.c. salt solution; a nodule appeared at the site of inoculation. There was cough which disappeared. The animal was killed at the end of 39 days; before death relative dullness measured 8.5 cm. and absolute dullness 4.6 cm.

Autopsy.—The right pleural cavity contains 39 c.c. of opaque fluid; the left cavity, 28 c.c. The parietal pleura is intensely injected and upon its surface are flat inconspicuous tuberculous nodules. In the mediastinum above the diaphragm extending far into the subpericardial membranes on each side is a very large firm tuberculous mass of pearly white color. The substernal lymphatic glands are greatly enlarged and continuous with a large tuberculous mass in the mediastinum situated immediately below them. The lungs contain a few scattered tubercles; tubercles are numerous in the liver.

Series G.—*Experiments with a more virulent tubercle bacillus.*—The foregoing experiments have been performed with a strain of tubercle bacillus of moderate virulence; this organism killed guinea-pigs in three or four weeks after intraperitoneal inoculation, but failed to kill rabbits when injected into the peritoneal cavity. In the following experiments a much more virulent organism which killed rabbits five weeks after intraperitoneal inoculation was used. Its virulence was well illustrated by the effect on dogs—death occurred with considerable rapidity, and instead of the gray-white sarcoma-like masses containing foci of caseation new formed tissue, which exhibited almost homogeneous caseation, was the result of fatal inoculation.

EXPERIMENT 29.—Dog, wt. 6,050 grm. Dullness over the right side of the chest increased during the week following inoculation and was reduced by the injection of leucocytes (Chart 16). The second injection produced no decrease, but the third caused material fall of the levels of relative and absolute dullness. Subsequent injections were not equally favorable. A nodule appeared in the chest wall at the site of inoculation and was injected four times with leucocytes (0.5 c.c.); it increased in size measuring 4.5 cm. across, and then diminished slightly. The animal became thin and weak and died after 39 days.

Autopsy.—The right pleural cavity contains 60 c.c. of serous slightly turbid fluid; the left cavity contains 35 c.c. of less turbid fluid. The pleural membranes are intensely injected. The mediastinum contains a flat mass of greenish caseous material (Fig. 6); a second mass just above the diaphragm extends into the left subpericardial membrane; the corresponding membrane on the right side is uniformly thickened by partly caseous tissue. The mediastinal lymphatic glands are enlarged and caseous. Distributed over the pericardium diaphragm and pulmonary surfaces are plaques of yellow tissue. The chest wall (at the site of inoculation) contains an ill-defined area of thickening and caseation and opposite in the substance of the lung is a round nodule of tuberculous tissue; the bronchial lymphatic glands on the right side are tuberculous. The lungs contain a few small miliary tubercles. The liver is enlarged and jaundiced and contains tubercles in immense number.

EXPERIMENT 30.—Dog, wt. 7,500 grm. Control. After inoculation thoracic dullness increased gradually (Chart 17); a small nodule formed in the skin at the site of inoculation. The animal died at the end of 47 days.

Autopsy.—The right pleural cavity contains 195 c.c. of yellow opaque fluid; the left, 150 c.c. The pleural membranes are injected more markedly on the right side; on the parietal pleura of the right side are flat yellow tubercles. The entire mediastinum is occupied by a greenish-yellow somewhat soft caseous mass merging into the greatly enlarged caseous substernal lymphatic glands. Both subpericardial membranes are greatly injected and contain large caseous masses (Fig. 7). Opposite the site of inoculation is a small caseous nodule 0.5 cm. across and extending about 1.5 mm. into the substance of the lung. The lungs contain no tubercles; in the liver are numerous miliary tubercles.

Tuberculosis was almost equally advanced in the two animals, although in that which received leucocytes tuberculous masses in the mediastinum and elsewhere were smaller. Wide dissemination in the injected animal is referable in part to inoculation of the lung. In the following experiment both injected animals and control were killed at the end of the same period and neither gave evidence that the lung substance had been entered by the tubercle bacilli with which they had been inoculated.

EXPERIMENT 31.—Dog, wt. 5,250 grm. A gradual increase of dullness followed inoculation (Chart 18); the first injection caused a rapid increase of dullness followed by decrease maintained until the next injection. This injection was followed by an increase which did not subside. There was accumu-

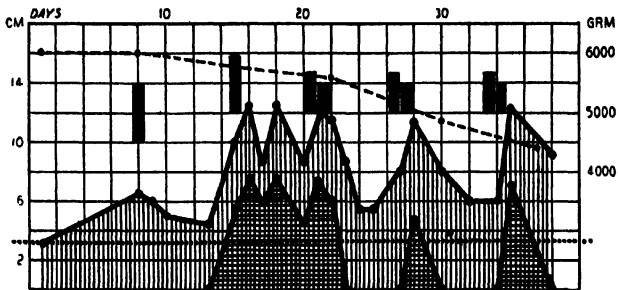


CHART 16. Experiment 29; injection of leucocytes.

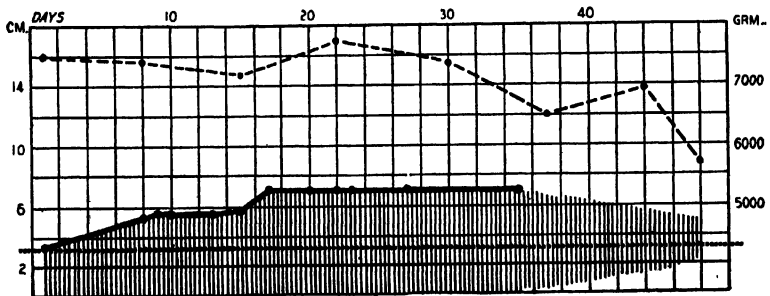


CHART 17. Experiment 30; control.

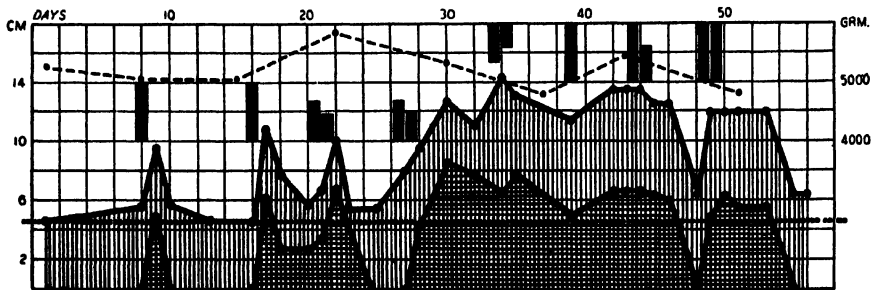


CHART 18. Experiment 31; injection of leucocytes.

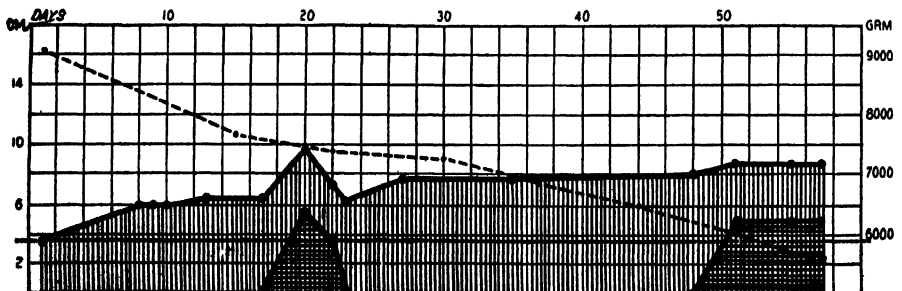


CHART 19. Experiment 32; control.

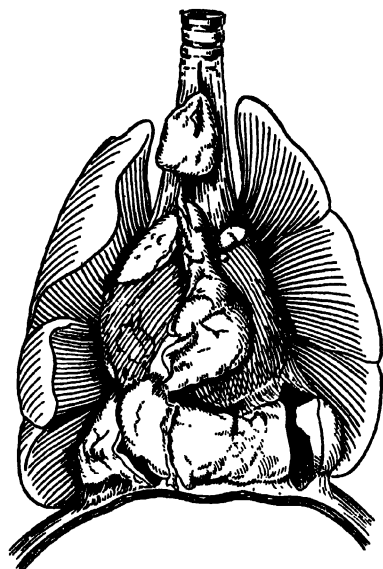


FIG. 6. Experiment 29; animal injected with leucocytes.



FIG. 7. Experiment 30; control.

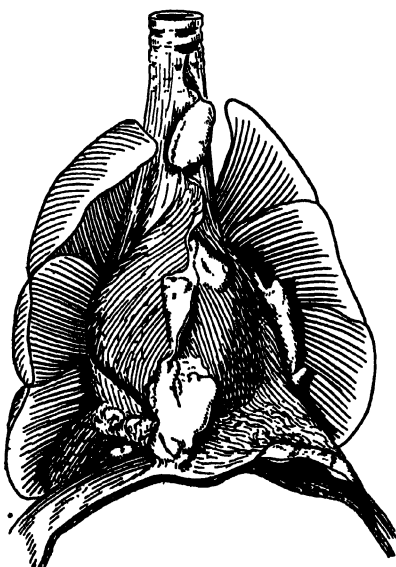


FIG. 8. Experiment 31; animal injected with leucocytes.

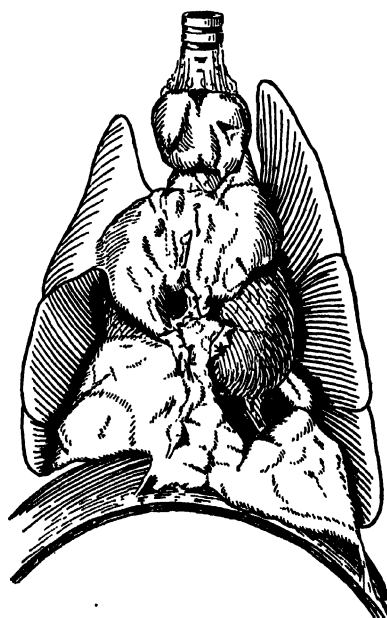


FIG. 9. Experiment 32; control.

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lation of a large pleural effusion and subsequent injections exhibited only occasional tendency to depress its level (fifth and seventh injection). A nodule formed in the chest wall during the third week after inoculation, increased in size and broke upon the surface; it was injected three times with leucocytes. During the seventh week of the disease a swelling appeared over the lower bone of the right hind leg and soon breaking upon the surface formed an ulcer with undermined edges about which there was redness of the skin. Leucocytes similar to those previously employed were applied to the wound and injected beneath its edges; a sterile dressing was put about the leg. Two days later when the bandage was removed swelling and redness had almost disappeared and the ulcer was clean and dry but exhibited no tendency to heal. The animal became thin and very weak and died 55 days after inoculation.

Autopsy.—The right pleural cavity contains 33 c.c. of deep yellow fluid; the left cavity, 14 c.c. of reddish fluid. The pleura of the diaphragm and chest wall is roughened by a thin irregularly distributed layer of fibrin and on both parietal and pulmonary pleuræ occur a few small tuberculous plaques. The mediastinum is thickened and contains small masses of friable caseous tissue from 1 to 3 mm. in thickness (Fig. 8). Similar thin plaques of caseous material with rough surface of somewhat eroded appearance occupy the subpericardial membranes. The substernal lymphatic glands are hard and caseous, 1.7 cm. in length. The lungs contain no tubercles recognizable by macroscopic examination. The liver is enlarged and contains tubercles. The spleen is large and soft, but exhibits no tubercles. Lymphatic glands in contact with the pancreas are enlarged and caseous; the mesenteric glands show the same alteration.

EXPERIMENT 32.—Dog, wt. 9,050 grm. Control. After inoculation thoracic dullness increased during three weeks (Chart 19), decreased and again increased; a nodule formed at the site of inoculation. The animal became thin and weak and conjunctival jaundice was present before it was killed, when almost moribund, 55 days after inoculation.

Autopsy.—The right pleural cavity contains 104 c.c. of almost clear, amber yellow fluid; the left pleural cavity, 116 c.c. The pleura is slightly thickened and upon the parietal and visceral surfaces, particularly on the right side, are numerous flat nodules. The mediastinum is replaced by a great mass of fairly soft, greenish yellow, in great part caseous tissue extending the whole length of the sternum and projecting outward into each subpericardial membrane (Fig. 9). The substernal lymphatic glands are greatly enlarged and caseous, measuring 2.3 cm. in length.

The lung on the surface and in its substance contains an immense number of tubercles. The liver is jaundiced and contains innumerable tubercles. The spleen is enlarged and contains thickly scattered opaque tubercles. Tuberculous glands of great size occur near the pancreas.

These experiments, in which a virulent tubercle bacillus was used, were undertaken under unfavorable conditions; dogs of approximately equal size were not obtainable and the dogs used as controls were much larger (9,050 and 7,500 grm.) than those which were injected (6,050 and 5,250 grm.). Fall of the level of thoracic

dullness frequently followed injection of leucocytes, but was less constant than in preceding experiments in which the less virulent microörganism was employed. It is noteworthy that for two injections in one animal (third and fourth injections in Experiment 29) and for one injection in the other (fourth in Experiment 31) cells were used which were obtained four and five days after injection of turpentine and were almost entirely necrotic. Nevertheless, although the injections failed to prolong life, the anatomical condition observed at autopsy showed that they had exerted an influence upon the development of the tuberculous lesion similar to that which was evident when somewhat less virulent microörganisms had been inoculated.

The quantity of fluid found at autopsy in the pleural cavities of the control animals has been far greater than that of the injected animals. The diagrams which show the size and distribution of tuberculous tissue indicate that the lesion has been more advanced in the controls, which contain throughout the mediastinum large masses of caseous tissue merging into one another (see Figs. 7 and 9). In the injected animals (see Figs. 6 and 8), especially in Experiment 31, tuberculous masses are less extensive and are of smaller size. In Experiment 30 the lung has been punctured and infected at the time of inoculation, so that there is no opportunity of comparing the effect of injections upon general dissemination of tuberculosis, but between Experiment 31 and its control, Experiment 32, in which the animals lived the same length of time, comparison is possible; tuberculosis in the lungs, liver and spleen was more advanced in the control than in the injected animal.

The almost constant effect of leucocytes injected into the pleural cavity of an animal from a week to ten days after intrapleural inoculation with tubercle bacilli is a fall of the level of thoracic dullness, elevated by the presence of effusion or of newly-formed tuberculous tissue. This fact is especially noteworthy because leucocytes in similar amount injected into the normal pleural cavity cause a rapid but temporary accumulation of fluid and increase of dullness over the dependent part of the chest. The first injection of leucocytes into eleven tuberculous animals was followed in seven instances by a fall of dullness recognizable twenty-four hours later;

in two experiments there was no depression of the level of dullness until forty-eight hours later. In only two animals did a reaction occur resembling that of the normal animal, namely, rise of dullness following injection with subsequent fall; in one instance this level finally fell below the level at time of injection, but in one instance it remained above the previous level.

Injections after the first exhibited a similar almost constant tendency to reduce the level of thoracic dullness; a preliminary elevation followed within two or three days by a fall below the level at the time of injection was frequently observed.

The relation of depression of thoracic dullness to diminution of pleural effusion is well shown by the amount of fluid present at autopsy in the chest of injected and uninjected animals. The following figures represent the quantity of fluid present in the chest of such animals dead or killed at approximately equal intervals after inoculation.

Injected Animal.			Control.		
Number.	Right.	Left.	Number.	Right.	Left.
Experiment 18	0 c.c.	0 c.c.	Experiment 20	2 c.c.	15 c.c.
23	12	11	24	235	184
25	0	0	26*	170	125
27	5.5	10	28	39	28
29	60	35	30	195	150
31	33	14	32	104	116

In one series of experiments (Series B) the animal which had received injections contained more effusion than the control; in this instance chronic non-tuberculous pleurisy not apparently referable to the tubercle bacillus had been caused by frequently repeated and very large injections of leucocytes. The experiment was performed at the beginning of the present study and accurate measurement of the quantity of fluid was not made.

Diminution of abnormal thoracic dullness caused by repeated injection of leucocytes into the tuberculous pleural cavity is doubtless due to diminution of tuberculous tissue in and about the mediastinum as well as to diminution of fluid. Evidence of the disappearance of such tissue is obtainable only by autopsy and in a number

* In this experiment leucocytes were injected into the pleural cavity twenty-four hours before death.

Animal Injected with Leucocytes.							Control.						
Number.	Duration of Disease.	Disseminated Tubercles on Pleural Surfaces	Tuberculous Masses in Mediastinum.	Length of Subperitoneal Lymphatic Glands.	Dissemination of Miliary Tubercles Elsewhere.	Remarks.	Number.	Duration of Disease.	Disseminated Tubercles on Pleural Surfaces	Tuberculous Masses in Mediastinum.	Length of Subperitoneal Lymphatic Glands.	Dissemination of Miliary Tubercles Elsewhere.	Remarks.
Exp. 23	32 days	Absent	Large	3.0 cm.	Liver Lungs	Puncture of lung	Exp. 24	32 days	Numerous	Large	3.1 cm.	Liver Lungs	Puncture of lung
Exp. 25	33 days	Absent	Absent	3.5 cm.	Liver Lungs		Exp. 26	33 days	Numerous	Large	3.5 cm.	Liver Lungs	
Exp. 27	39 days	Present in small number	Large	2.3 cm.	Liver		Exp. 28	39 days	Present in considerable number	Large	3.0 cm.	Liver Lungs	
Exp. 29	39 days	Numerous	Large	2.0 cm.	Liver Lungs	Puncture of lung	Exp. 30	47 days	Numerous	Large	3.0 cm.	Liver	
Exp. 18	50 days	Present in small number	Small	1.6 cm.	Liver Lungs Kidneys		Exp. 20	50 days	Numerous	Mod-erately large	1.2 cm.	Liver Lungs Kidneys	
Exp. 31	55 days	Present in small number	Small	1.7 cm.	Liver		Exp. 32	55 days	Numerous	Large	2.3 cm.	Liver Lungs Spleen	
Exp. 9	56 days	Absent	Small and fibroid	1.4 cm.	Liver Lungs Kidneys		Exp. 10	56 days	Numerous	Large	2.1 cm.	Liver	

THE ANTAGONISTIC ACTION OF CALCIUM UPON THE INHIBITORY EFFECT OF MAGNESIUM.

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INTRODUCTORY.

CALCIUM and magnesium are chemically closely related bodies. They are also close companions in the fluid and solid tissues of the animal body. It seems to be a generally accepted opinion that both alkali earths exert influences in the animal body which run in a similar direction and are of a similar character. This frequently goes even so far as to assume that both ions are capable of substituting one another in the functions of the body. While there was a great deal of work done in recent years on the antagonism existing between the effects of calcium, sodium, and potassium (Ringer, Locke, Howell and his pupils, Jaques Loeb and his pupils, Overton), there was very little work done on the possible differences between the effects of calcium and magnesium, not to speak of an antagonism between them.

In speaking of the effects of calcium and magnesium we intend to deal here only with their relations to nerve and muscle. Here we encounter in the foreground the important and extensive work done by J. Loeb and his pupils. J. Loeb¹ put forward in 1899 the view that calcium and magnesium are ions which inhibit rhythmical muscular contractions. In studying the rhythmical twitchings of the frog gastrocnemius immersed in solutions of sodium chloride (and allied alkali compounds), Loeb discovered that these rhythmical contractions can be inhibited by the addition of compounds of po-

¹ J. LOEB in FICK'S Festschrift, republished in "Studies in general physiology," Chicago, 1905, ii, p. 518.

tassium or calcium. It was then found further that "the entire group of (alkali earths) Be, Mg, Ba, Sr, and also Mn and Co" are capable of inhibiting these rhythmical contractions. Barium was later eliminated from this group, as it was found to cause contractions rather than to inhibit them. It should be stated that, in this first paper as well as in the many subsequent papers by Loeb himself and also by his many pupils, it was the inhibitory effect of calcium which was extensively studied and discussed. The possible inhibitory effect of magnesium upon muscular contractions was touched upon only occasionally and in a perfunctory way, while at the same time in the studies upon artificial parthenogenesis $MgCl_2$ received foremost attention. However, in the recent writings of Loeb and his pupils the inhibitory effect of magnesium salts is brought out more prominently, although some² state expressly that "calcium inhibits more strongly than magnesium."³

Within the last few years we made several communications on the effects of magnesium salts.⁴ They were all based on the hypothesis that magnesium favors inhibitory processes in the animal body. Some of the essential facts reported by us were that subcutaneous or intravenous injections of magnesium salts cause a state of anesthesia and paralysis from which, if the dose be not too large, the animal may completely recover.

The starting-point for our hypothesis was the observation, made several years before the first communication, that an intracerebral injection of two or three drops of magnesium sulphate caused a peculiar paralysis of the animal, while the injection of other salts was either indifferent or caused convulsions. This observation was

² BANCROFT: *The journal of biological chemistry*, 1907, iii, p. 209.

³ The following variations of the same statement might perhaps serve as an illustration. In the first article (FICK's *Festschrift*, 1899) we read: "We are therefore indebted to the calcium contained in our blood for the fact that our muscles do not twitch continually" (quoted from "Studies in general physiology," part ii, p. 530, footnote). "It is due to the presence of Ca- (and K-) ions in our blood that our muscles do not contract rhythmically" (*This journal*, 1900, iii, p. 328). "As I stated six years ago, we owe it to the Ca- and Mg-salts in our blood that our skeletal muscles do not contract rhythmically like our heart" (*The dynamics of living matter*, 1906, p. 79).

⁴ MELTZER and AUER: *This journal*, 1905, xiv, p. 366; 1906, xv, p. 387; 1906, xvi, p. 233; 1906-1907, xvii, p. 313. MELTZER: *Medical record*, 1905, lxviii, p. 965. MELTZER and HAUBOLD: *Journal of the American Medical Association*, 1906, xlvi, p. 647. MELTZER and AUER: *Journal of experimental medicine*, 1906, viii, p. 692.

demonstrated to the American Physiological Society at the New Haven meeting, 1899.⁵

While we are thus in full agreement with J. Loeb in regard to the inhibitory effect of magnesium, we could never persuade ourselves to an acceptance of the view that calcium also was a pure inhibitory factor. We are further not sure whether or not our definition of inhibition coincides with the one entertained by Loeb and his pupils. In our papers we variously took occasion to define the meaning of inhibition as we use it. It is an inhibition as exemplified by the vagus inhibition of the heart or the splanchnic inhibition of intestinal peristalsis. It is, then, not simply the prevention of a rhythmic movement, or the suppression of any vital process; it is the reduction or abolition of the irritability of the contractile tissues, including, of course, also the abolition of clonic or tonic contractions, that is, the production of a *relaxation* of the concerned muscular tissues. From this point of view we could never think, as Loeb's pupils do, of looking upon the reduction or suppression of diuresis, upon the suppression of glycosuria, or upon the prevention of hemolysis by calcium salts, as inhibitory phenomena. These suppressions might have been induced by some active, exciting, and not by an inhibitory process. Furthermore, it does not seem to us that the suppression of muscular twitching on a certain occasion is an all-sufficient fact for the consideration of calcium as a general inhibitory agent. It may still be an exciting agent at other times. Potassium, for instance, which, according to Loeb, suppresses the twitchings just as well as calcium, causes, according to Zoethout,⁶ a pupil of Loeb, a tonic contraction of the frog's gastrocnemius muscle, which is surely an exciting process. Moreover, this tonic contraction caused by potassium can be inhibited by sodium chloride (Zoethout). Should we consider sodium chloride also an inhibitory agent?

⁵ MELTZER: This journal, 1900, iii, Proceedings of the American Physiological Society. In our first communication on the effects of magnesium salts, where we have given some references to the literature on that subject, we inadvertently omitted to mention LOEB'S view of the inhibitory effect of magnesium. We regret this oversight. It was caused by the fact that until that time LOEB himself discussed essentially and extensively the inhibitory effect of calcium, so that his casual brief references to magnesium entirely slipped our mind. We may mention here also that, according to LEE, *The microtometist's vademecum*, 4th ed., 1896, p. 16, TULLBERG (1892) and RADENBAUGH (1895) suggested the employment of magnesium salts for the anesthesia of certain invertebrate animals.

⁶ ZOETHOUT: This journal, 1902, vii, p. 199.

Again, calcium itself decreases the irritability in one set of instances, in another set it unquestionably increases the irritability of muscle and nerve, and we do not need for this purpose to draw the mechanism of the heart beat into the discussion. The paralyzing effect of potassium, lithium, rubidium, and caesium salts upon muscle and nerve trunks can be promptly neutralized by the addition of calcium chloride (Overton⁷). The restoration of the lost irritability in these cases is surely a phenomenon just opposite to inhibition. Furthermore, the abolition of indirect irritability by sodium chloride (Carslaw,⁸ Locke,⁹ Cushing,¹⁰) and the "curare-like effects" of the salts of potassium, rubidium, ammonium, and caesium (Overton¹¹), is promptly corrected by the addition of calcium. Here, again, calcium exerts a distinctly exciting and not an inhibitory effect upon the motor nerve endings.

In harmony with these facts was our own experience with calcium in its effect upon living mammals. We may mention it here briefly. Except for the inhibitory effect upon intestinal peristalsis (J. B. MacCallum) *we could never produce by subcutaneous or intravenous injections of calcium salts an anesthetic or paralytic effect in any way similar to that produced by magnesium salts. Even when large fatal doses were employed by intravenous injections, the animal was wide awake and the lid reflex, etc., was preserved until shortly before death.*

On the basis of all these facts we could not persuade ourselves that the various interesting and important phenomena observed by Loeb and his pupils upon the effects of calcium justified the positive assumption that calcium is an exclusively inhibitory agent. Moreover, we could not agree with the assumption, made by many others besides Loeb and his school, that calcium and magnesium are exerting a similar effect upon animal tissues. Our own studies¹² brought out various facts showing a striking contrast between the effects of these two chemical elements.

The continuation of the last-mentioned studies led up, finally, to the discovery that calcium is rather the strongest antagonist to the

⁷ OVERTON: Archiv für die gesammte Physiologie, 1904, cv, p. 176.

⁸ CARSLAW: Archiv für Physiologie, 1887, p. 429.

⁹ LOCKE: Centralblatt für Physiologie, 1894, viii, p. 166.

¹⁰ CUSHING: This journal, 1902, vi, p. 77.

¹¹ OVERTON: *Loc. cit.*

¹² MELTZER and AUER: Journal of experimental medicine, 1908, x, p. 45.

inhibitory effects of magnesium. The report of these observations forms the subject of this paper.

Before entering upon the report we wish, however, to make the statement that Loeb¹³ himself recently reported certain facts showing the existence of an antagonism between calcium and magnesium in their effects upon a jelly-fish (*Polyorchis*) found on the Pacific coast, and that Anne Moore,¹⁴ a pupil of Loeb, reported several years before that the poisonous effect of a pure $MgCl_2$ solution upon a fresh-water fish (trout) can be neutralized by the addition of $CaCl_2$.

Alfred G. Meyer¹⁵ states that calcium assists sodium chloride in counteracting the inhibitory effect of magnesium which the latter exerts upon the movements of *Cassiopea*.

EXPERIMENTS ON THE ANTAGONISTIC ACTION OF CALCIUM TO THE INHIBITORY EFFECTS OF MAGNESIUM.

Methods. — The observations to be reported in this paper were made essentially on rabbits. The solutions of magnesium salts were given either subcutaneously or intravenously; the calcium salts were given in all cases intravenously, in some cases through the ear vein, in others through a cannula inserted into one of the external jugular veins. Of the various magnesium salts there were used: the sulphate, chloride, nitrate, and acetate; of the calcium salts: the chloride, nitrate, and acetate. For the subcutaneous injection of magnesium, $m/1$ concentrations were used in all cases. In the intravenous experiments the magnesium salts were used in various concentrations, mostly in $m/8$ solutions. The calcium salts in all cases were $m/8$. The influence of these salts upon respiration, blood pressure, cardiac vagus, etc., were studied in numerous experiments by the graphic method. Also stimulations of the peripheral and central ends of a sciatic nerve were made for the purpose of studying the antagonistic effects under discussion upon the motor and sensory functions.

¹³ LOEB: *Journal of biological chemistry*, 1905-1906, i, p. 427. *

¹⁴ ANNE MOORE: *This journal*, 1901, iv, p. 391.

¹⁵ ALFRED G. MEYER: *Rhythmical pulsation in Scypho-medusæ*, Carnegie Institution of Washington, 1906, p. 46.

In all experiments which required cutting, ether was used freely during the time of operation.

Experiments with subcutaneous injections of magnesium salts. — In our first paper on general anesthesia due to magnesium salts, we stated that for magnesium sulphate in subcutaneous injections the anesthetic dose is between 1.25 and 1.75 gm. per kilo. (The water of crystallization is included in all our figures.) Doses exceeding 1.75 per kilo were usually fatal. For MgCl_2 the dose was even lower. With an anesthetic dose, thirty to forty minutes after a subcutaneous injection, the animal would usually lie perfectly limp without any lid reflex and without a reaction to a stimulation. When the dose exceeded the safety point, the respiration would cease pretty early after the injection, the cardiac activity usually persisting longer than the respiration.

In our present series we usually employed fatal doses of the magnesium salts, and calcium was injected at various stages after the onset of a pronounced effect. We shall illustrate our results with a few abbreviated protocols.

Experiment 1. — Gray male rabbit, 1550 gm.

10 A. M. Injected subcutaneously, middle of abdomen, 13 c.c. MgCl_2 in $m/1$ solution = about 2 gm. per kilo.

10.30. Respiration very shallow, slow, practically no lid reflex, limp. Injected through ear vein 8 c.c. CaCl_2 in $m/8$ solution. *Respiration at once deepened and quickened, animal turned over and sat up.*

10.50. Again under the influence of magnesium, lying on side, but respiration good. Injected again 6 c.c. of CaCl_2 $m/8$ through ear vein. Animal turns over, sits up, and remains well.

Two grams per kilo of MgCl_2 is a surely fatal dose; the animal was completely paralyzed and already very near a fatal issue. The injection of CaCl_2 immediately improved the respiration and reversed the paralysis.

Experiment 2. — Gray female rabbit, 1210 gm.

11.10. Injected subcutaneously in the right side of abdomen 10 c.c. MgCl_2 in $m/1$ solution; slight loss; added 2 c.c. of the solution; altogether more than 2 gm. per kilo of MgCl_2 .

11.40. Lid reflex gone, respiration slow and shallow, completely relaxed, no response on pinching tail or leg.

Injected through ear vein 6 c.c. $m/8$ CaCl_2 . After 2 c.c. respiration deepened and quickened; after finishing the injection lid reflex active and animal turns over and sits up.

12.10. No lid reflex again, respiration shallow. Again given through ear vein 6 c.c. $m/8$ CaCl_2 . Same effect as before, animal sits up and remains normal.

Experiment 3. — White rabbit, 1450 gm. Animal on holder, ether given, and a cannula inserted into the left jugular vein.

10.15. Injected subcutaneously 14.5 c.c. MgSO_4 in $m/1$ solution = 2.5 gm. per kilo.

10.35. Respiration rapid and shallow, lid reflex reduced.

10.42. Respiration shallow and labored, legs limp, lid reflex slight.

10.44. Started calcium acetate, running slowly from burette into external jugular vein. Respiration improved at once, is deeper and more rapid, *hind legs become stiff*.

10.50. 10 c.c. ran in. Lid reflex active, respiration good. Strong struggle on pinching toes.

12.00. Respiration and heart good, but lid reflex reduced again and only moderate motion on pinching tail.

1.15. Breathes easily, moves occasionally with some vigor.

3.00. Wound sewed up and animal taken from table. No further trouble.

On account of the large dose of MgSO_4 and the occasional failure of the ear vein method, when it is most urgently needed, a cannula was inserted into the external jugular vein. Although the dose of MgSO_4 in this experiment was quite large, the single dose of 10 c.c. $m/8$ calcium acetate was sufficient to bring about complete recovery of the animal without sequela. A noteworthy incident is *the stiffness of the legs after the introduction of CaCl_2 , a sharp contrast to the paralysis just a few minutes before*.

Experiment 4. — White and black male rabbit, 1070 gm. Animal on holder, ether given, and cannula inserted into the external jugular vein.

12.05. Injected subcutaneously in the epigastric region 13 c.c. MgSO_4 in $m/1$ solution = 3 gm. per kilo.

12.38. Lid reflex nearly gone, breathing very shallow, some slight, short twitchings (asphyxia?).

12.40. Lid reflex completely gone, animal completely paralyzed, no response on pinching tail, respiration very shallow.

12.45. Respiration hardly perceptible, heart good. Started $m/8$

$\text{Ca}(\text{CH}_3\text{COO})_2$ from burette very slowly. Respiration immediately deeper and more rapid.

12.47. 2 c.c. of the calcium acetate ran in so far. Lid reflex returned, sensation present, struggled twice.

12.50. Legs stiff, left extended, right flexed, but resistant to motion.

12.55. 10 c.c. in; stopped the infusion.

1.05. Respiration, heart, lid reflex good. Animal attempts to free itself.

2.45. Respiration, heart, sensation good, lid reflex less prompt. Given again 4 c.c. calcium acetate and animal taken from board; uneventful recovery.

In this experiment the rabbit recovered from a dose of 3 gms. MgSO_4 per kilo, and again the one dose of 10 c.c. calcium acetate was practically sufficient to bring about permanent recovery; and here again there was stiffness of the legs after the use of calcium acetate.

Experiment 5. — White male rabbit, 1530 gm.

11.05. Injected subcutaneously 12.5 c.c. magnesium nitrate in $m/1$ solution = 2 gm. per kilo.

11.25. Lying on side, slow and shallow respiration, no lid reflex.

Injected through ear vein 7 c.c. calcium nitrate in $m/8$ solution. Immediately after finishing injection animal sat up and moved around. Respiration excellent.

11.45. Lying on side again, no response to pinching tail or leg, respiration shallow, and no lid reflex. 1 c.c. calcium nitrate given through ear vein. Animal sat up.

11.55. Lying on side again. Attempt made to give again calcium nitrate by ear vein, but was unsuccessful.

2.05. Lying on side still, urinated a good deal; respiration slow, but fairly deep. Responds with movements of extremities to pinch of tail. (Animal found dead next morning, death apparently being due to perforation of the colon produced in an attempt to wash it out.)

Experiments like the above were made in large number and practically without a single failure. As long as there were some efficient heart beats and a few gasps of respiration, the intravenous injection of a solution of calcium chloride infallibly improved the respiration instantaneously and revived the animal. As can be seen from

the above protocols, with the assistance of the calcium injections animals survived even such large fatal doses as 3 gm. per kilo of a magnesium salt. When the quantity of the subcutaneously injected magnesium salt exceeded the fatal dose perceptibly, frequently a single injection of a calcium salt would not be sufficient to establish the recovery permanently. After a sudden and apparently complete awakening from the deadly influence of the magnesium, the animal would gradually sink for a second time into a comatose state. A second injection would again completely reawaken it. In most cases such a second injection was apparently unnecessary. If for some reason the second injection was not made, the animal, as a rule, finally recovered anyhow. Without the second injection the animal would lie in an anesthetic and relaxed state for hours, but the respiration seemed not to be in danger. Here was the significant difference between the anesthetic state before the first injection of the calcium and the similar state which set in again after this injection: that the first primary state was progressive, full of immediate danger for the respiration, while the second state was at the worst stationary, and with a tendency to regression, the respiration being at no time in real danger.

The reason for the relapse into the secondary state seems to be simply this: the intravenously injected calcium is capable of neutralizing of course only that part of the magnesium which it finds already absorbed from the subcutaneous tissues. But since the magnesium continues to be absorbed after the injection of the calcium, especially when the injected magnesium dose was large, a new state of anesthesia is liable to come on. However, the effect of the magnesium upon the respiratory centre seems to be easily kept in check even by a very small dose of calcium, and such a small dose seems to remain active for some time after an intravenous injection of that substance, as will be seen in experiments to be described later. Hence the favorable condition in the secondary state of anesthesia which sets in after an injection of calcium.

A large dose of calcium is sometimes sufficient to neutralize efficiently all the depressing effects of the subsequently absorbed magnesium, as can be seen from Experiments 3 and 4, in which a single injection of 10 c.c. calcium was sufficient to overcome a magnesium dose of 2.5 or 3 gm. per kilo.

From these considerations it follows that the later the injection of calcium is given, the better the chance of neutralizing a larger

part of the absorbed magnesium. On the other hand, we have to record the fact that the danger of respiratory paralysis can be obviated by an injection of only a small quantity of a calcium salt, as we have learned in experiments which were prepared for a demonstration or for photographing. At the approach of danger an injection of 1 c.c. of calcium will keep the animal out of danger for some time, while it will remain in a complete state of anesthesia. Fig. 1 illustrates the last-mentioned condition. The animal received



FIGURE 1. — Rabbit under the influence of 2 gm. $MgCl_2$ (crystals) per kilo body weight, given subcutaneously in $m/1$ solution. In order to prevent respiratory paralysis from this lethal dose 1 c.c. of $m/8$ $CaCl_2$ had been injected into the ear vein. Note complete relaxation of animal.



FIGURE 2. — Same animal less than one minute after an intravenous injection (ear vein) of 6 c.c. $m/8$ $CaCl_2$. Animal able to move about easily.

$MgCl_2$ subcutaneously 2 gm. per kilo for the purpose of having it photographed before and after the injection of calcium. However, twenty-five minutes after the injection the animal was very comatose, bordering closely on the danger line, while the arrangements for photographing were not yet ready. The animal received then 1 c.c. of $CaCl_2$ $m/8$ through the ear vein. It remained then for over half an hour in a stationary state of deep anesthesia, at the end of which time it was photographed. The picture shows the complete relaxation of the entire animal, which could be placed in any position; it shows also the clamp upon the ear margin, where the injection was given. Fig. 2 shows the same animal about one minute after an injection of 6 c.c. of $CaCl_2$ in the marginal vein of the same ear in which the first injection was given.

When the quantity of magnesium injected was not much more than the maximum anesthetic dose, an intravenous injection of a few c.c. of a calcium salt in $m/8$ solution restored the animal permanently to a normal state. A number of rabbits received, at intervals of one or two days, several injections of magnesium and calcium salts without any noticeable detriment.

We shall cite here one more protocol of an experiment on a monkey.

Experiment 6. — Female monkey (*Macacus rhesus*), 2200 gm.

12.00 M. Injected subcutaneously, front of thorax, 9 c.c. of MgSO_4 in $m/1$ solution = 1 gm. per kilo, and returned animal to the cage.

1.00. Found perfectly limp in cage, only occasional very shallow respiration; no lid reflex; heart beating well. A cannula was hurriedly introduced into the right femoral vein and 2 c.c. calcium acetate in $m/8$ solution injected. At once respiration became deep and rapid, lid reflex active; moved legs. Animal remains on holder.

1.40. Respiration fair, but with active expiration; does not follow objects with the eyes.

1.42. Injected again through femoral vein 2 c.c. $m/8$ calcium acetate. Respiration at once deepened, became more rapid, and less expiratory in character; lid reflex active; no change in heart rate. Follows movements of people with the eyes, moves head; no movements of arms and legs. Remained in about the same condition until about 4.30, when she began moving arms and legs.

4.55. Shows fight now, opens mouth when approached.

5.30. Wound sewed up, attempts to escape when removed from holder. Sits up in cage, moves about; completely recovered.

The injection was given probably into the pectoral muscle, or at least subfascially, as in purely subcutaneous injections 1 gm. per kilo has never such a profound effect upon monkeys. The neutralizing effect of the calcium injection was as striking here as in rabbits.

In the foregoing experiments we learned that *the intravenous injection of any calcium salt is capable of neutralizing nearly instantaneously all the symptoms produced by the subcutaneous injection of any magnesium salt*. We hardly need to state expressly that the intravenous injection of calcium will not neutralize all doses of magnesium. We did not go further in the subcutaneous injections of magnesium salts than 4 gm. per kilo. In this case the calcium injections relieved temporarily the symptoms, but the heart became irregular and failed ultimately, after a good deal of calcium was given.

Experiments with intravenous injections of magnesium. — We shall now relate some of our observations made in experiments in which both salts were given slowly intravenously.

Experiment 7. — White female rabbit, 2650 gm. Ether; cannulas introduced into both external jugular veins and connected with burettes, one containing MgCl_2 in $m/2$ solution and the other containing CaCl_2 in $m/8$ solution; tracheotomy; a pleural cannula introduced into the left pleural cavity and left lung redistended; cannula connected with a Marey tambour to record the respiration.

After the animal recovered sufficiently from the ether, MgCl_2 was run into the jugular vein fairly slowly, about 1 c.c. in two minutes. After nine minutes, when about $4\frac{1}{2}$ c.c. had been infused, the animal became asphyxiated and artificial respiration was started. The infusion of MgCl_2 continued. About a minute later the artificial respiration was discontinued for twenty-four seconds and no sign of spontaneous respiration appeared, the writing-lever marking only the heart beats. The artificial respiration was resumed. It was discontinued again after forty-five seconds, and thirty-two seconds later, after no sign of a spontaneous respiration had appeared, the infusion of $m/8$ CaCl_2 into the other jugular vein began, while the infusion of $m/2$ MgCl still continued. A few seconds later the first spontaneous respiration appeared, and within twenty seconds, when not much more than half a cubic centimetre of the calcium solution had entered, the respiration was quite normal again. (The tracings in Fig. 3 illustrate the essential points of the above statement.)

This experiment demonstrates that a minute quantity of a dilute solution of calcium chloride is sufficient to practically instantaneously restore the respiration inhibited by 7 c.c. of $m/2$ magnesium chloride. However, we have to add that in this instance after the first appearance of asphyxia less than 2 c.c. magnesium was given and less than four minutes' time passed before the infusion of the solution of the calcium salt was begun. The neutralizing effect of the calcium loses its promptness in proportion with the increase of the quantity of magnesium salts infused and with the prolongation of the interval passing between the appearance of asphyxia and the beginning of infusion of the calcium solution, so that a condition may finally present itself when the administration of calcium may remain with very little or even with no effect.

Experiment 8. — Black female rabbit, 1480 gm. Ether; cannulas in both external jugular veins; tracheotomy; left sciatic exposed, intact, placed in a Ludwig electrode and connected with an induction coil (Porter); ether discontinued.

12.05. Start infusion of $m/8$ $MgCl_2$ into left jugular vein. Stimulation of sciatic with 100-coil distance gives a strong contraction of the left leg as well as a general reaction.

12.13. 17 c.c. ran in, lid reflex gone, respiration very shallow.

12.14. Practically no respiration, starting artificial respiration.

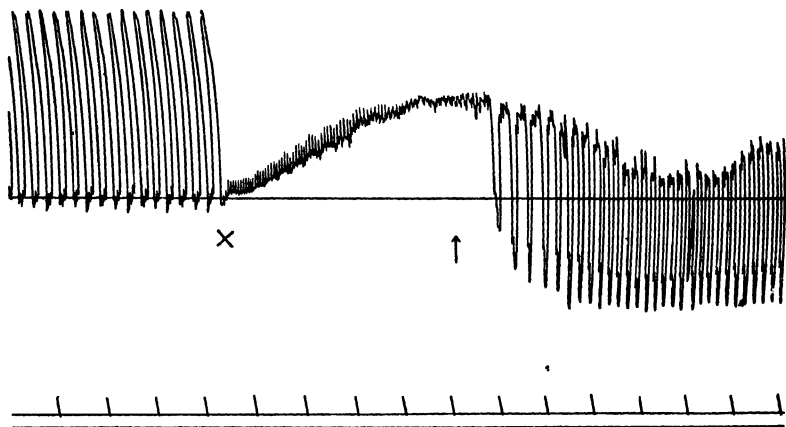


FIGURE 3.—Respiratory tracing taken by means of a cannula from the pleural cavity. The horizontal line cutting the tracing marks atmospheric pressure: above the line is positive pressure, below the line is negative pressure. Time, six-second intervals. The beginning of the curve shows artificial respiration, spontaneous respiration having been abolished by $m/2$ $MgCl_2$ still flowing in; at the point marked X artificial respiration was discontinued, and the curve now shows volume changes due to the heart beats. For over thirty seconds no spontaneous respirations appeared; then (at the arrow-mark) $m/8$ $CaCl_2$ was infused slowly into the jugular vein, $m/2$ $MgCl_2$ still running into the other jugular vein at the original rate. After less than six seconds spontaneous respirations appeared in normal strength and rate (the down stroke in this part of the curve marks inspiration) and negative pressure was largely re-established in the pleural cavity after less than 1 c.c. of Ca had entered the circulation.

12.15. 21 c.c. infused. Sciatic 100: left toes move, also right reflexly, but no pain.

12.17. Sciatic 100: very slight contraction of left toes, no reflex to right.

12.21. Began infusion of $CaCl_2$ $m/8$ into right jugular vein; 4 c.c. ran in fairly rapidly. ($MgCl_2$ continued.) Respiration began at once. Sciatic 100: good contraction of left hind leg, strong reflex to right hind leg and also to front legs.

In this experiment spontaneous respiration was abolished by the magnesium solution long before a great reduction of irritability of the sciatic nerve to electrical stimulation was obtained, and here again

the irritability for afferent impulses was affected earlier than for motor impulses. Both, however, were reduced nearly to zero, at least for the original strength of stimulus, and the irritability of both recovered fairly well soon after the infusion of 4 c.c. $m/8$ CaCl_2 .

In some of the experiments of the kind last quoted, after the first recovery of the animal, the experiment was repeated by again bringing the animal under the influence of magnesium, and attempting to attain at least a temporary recovery by a second infusion of a calcium solution. In these instances it was often noticed that it now took a much larger dose of the magnesium salt to completely abolish respiration than on first trial, and that now the motor and sensory functions, as tested by stimulations of the sciatic nerve, succumb to the influence of magnesium more readily than the respiratory mechanism, — a condition which is the reverse of that which usually takes place at the primary experiment. *Apparently the respiratory mechanism, which becomes affected by magnesium more readily than any other function, is also more easily protected against this influence by calcium.* In the secondary experiments, therefore, the remainder of the calcium (within the blood or the tissues?) from the previous infusion is still able to keep up the resistance of the respiratory mechanism against the renewed attack of the magnesium.

The behavior of the motor function of the sciatic nerve with reference to the reversing effect of calcium was not as simple and uniform as that of the other functions, for instance as that of the lid reflex or the respiration. In the first place the degree and the rapidity of recovery was variable. While it was prompt and complete in one case, it was slow and insufficient in other cases. In two instances there was practically no recovery. However, in these cases also the calcium infusion evidently stopped the progress of the paralysis, although the infusion of the magnesium solution still continued. It must be stated, further, that in these cases the sciatic nerve was cut, and we thought it possible that, on account of the vaso-dilatation hereby produced in the muscles of the leg, more magnesium accumulated there and the inhibitory effect was therefore more profound; against this the incoming calcium was less efficient than against the inhibitory effect upon the other organs which harbored a smaller quantity of magnesium.

It should be remembered, however, that this unsatisfactory behavior of the motor apparatus of the leg applies only to the artificial conditions as we create them by stimulating the motor nerve electri-

cally. The normal motor impulses of the leg show the same prompt reversibility as the other functions. An animal which was completely paralyzed by magnesium, on receiving calcium, struggles with the legs as readily and as promptly as it breathes and winks.

We append the following greatly abbreviated protocol for the purpose of showing the inefficiency of calcium after the previous administration of a large dose of magnesium.

Experiment 9. — Gray rabbit, 1490 gm. (prepared as in Experiment 8, except that a Petzold induction coil was used). Sciatic 110: moderate general effect (motor, reflex, pain).

10.56. Began infusion of $m/8$ $MgCl_2$.

11.01. 21 c.c.; respiration practically nil; started artificial respiration.

11.07. 38 c.c. ran in (more than 3 c.c. a minute!).

11.08. Sciatic 110: no effect whatsoever. (Slight lid reflex present?!)

11.10. 46 c.c. $m/8$ $MgCl_2$. Started calcium acetate $m/8$.

11.11. 6 c.c. calcium acetate in; no respiration.

11.13. 49 c.c. magnesium and 19 c.c. calcium. No respiration and sciatic 110 negative. Stopped magnesium.

11.15. 30 c.c. calcium acetate ran in. No respiration; heart stopped beating, no recovery.

The infusion of calcium in this case had not the slightest effect; it perhaps even hastened the death of the animal. Here 46 c.c. of $m/8$ $MgCl_2$ were infused before calcium was started. In other instances in which 33 or 35 c.c. of a magnesium solution ran in before calcium was given, a final recovery was obtained, but its onset was late and it progressed slowly. This simply means that not all quantities of magnesium and not all stages of its poisonous effects can be overcome by calcium, — a fact to be expected *a priori*.

Experiment 10. — White male rabbit, 2200 gm. Ether; cannulas into both jugular veins and in carotid artery, the latter connected with a mercury manometer; a saturated solution of sodium sulphate used as connecting fluid; left vagus cut; Petzold coil with two Daniell cells used; the left burette filled with $MgCl_2$ in $m/2$ solution and the right burette with $CaCl_2$ in $m/8$ solution.

The blood pressure was about 125 mm. The secondary coil at 85 mm. distance was the minimal stimulus for the peripheral end of the vagus which stopped the heart (Fig. 4a).

The infusion of $m/2$ $MgCl_2$ began, and continued very slowly,

about 1 c.c. in eighty seconds. The blood pressure very soon began to fall slightly, but continuously. When 2 c.c. of the magnesium solution was in, stimulation with 85-coil distance did not completely stop the heart. When a little over $2\frac{1}{2}$ c.c. was infused, asphyxia set in and artificial respiration was started. Soon after, when about 3 c.c. of the solution had run in, 85-coil distance caused only moderate slowing of the heart and slight fall of pressure. Still later, after 4 c.c., even 50-coil distance had no effect. After 6 c.c. 40-coil distance failed to stop the heart. Now, while the magnesium solution continued to run in, the infusion of $m/8$ CaCl_2 began. The blood pressure, which had sunk to about 40 mm., immediately began to rise, and about fifty seconds later not only a stimulation with the secondary coil at 40, but even with a coil distance of 100, stopped the heart completely; and even with a coil distance of 120 mm. there was a distinct effect upon the heart beat. The blood pressure rose to about 70 mm. after only 1 c.c. of the calcium solution (see Fig. 4 b).

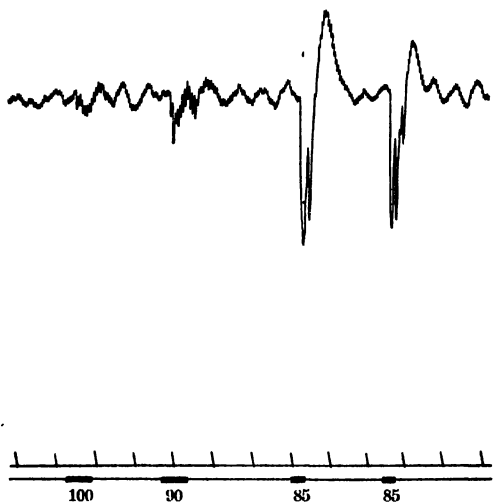


FIGURE 4 a. — Five sixths the original size. Normal blood-pressure tracing from the carotid artery of a rabbit showing minimal effective stimulus for the left peripheral vagus. Time in six-second intervals; this line also marks atmospheric pressure; the lowest line shows the duration of stimulation.

In this experiment we learn, first, that magnesium salts in concentrated solution reduce the cardio-inhibitory effect of the vagus. We learn further that this depression of the irritability of the vagus can be promptly removed by a relatively small dose of calcium and, what is more, the irritability becomes stronger than it was before the use of the magnesium. Before the magnesium was started, the heart was stopped by a stimulation of the vagus, when the secondary coil was at a distance of 85 mm. When it was at 90, it had practically no effect. After some calcium was infused, not only at 90 and at

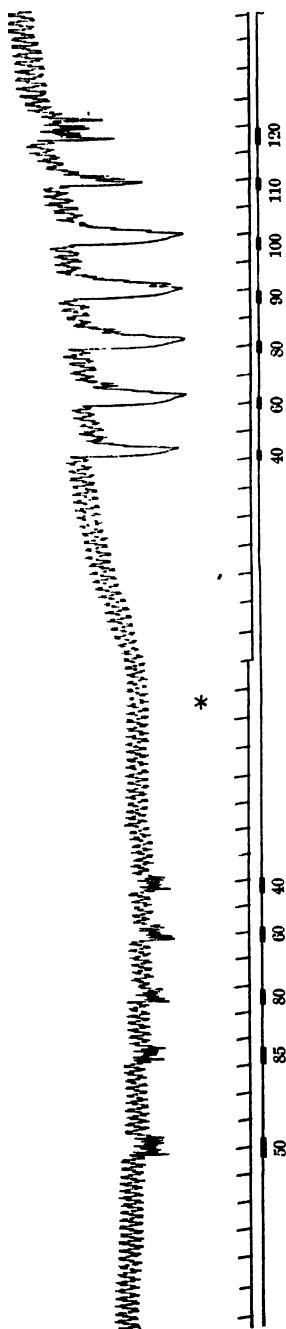


FIGURE 4 b.—About seven tenths the original size. ; Blood-pressure tracing from the same animal (Figure 4 a) under the influence of $m/2 \text{ MgCl}_2$. Artificial respiration. The lowest line marks the duration of stimulation. Time in six-second intervals; the time line also records the atmospheric pressure. At * $m/8 \text{ CaCl}_2$ was injected slowly into jugular vein, $m/2 \text{ MgCl}_2$ still being infused into the other jugular vein at the original rate. Note the rise in blood pressure following immediately upon the entrance of CaCl_2 into the circulation, and the greatly increased effectiveness of left peripheral vagus stimulation. Before the injection of calcium, stimulation with the secondary coil at 40 produced only a slight effect; after the calcium, stimulation with the coil at 120 showed a definite fall in blood pressure.

100 did a stimulation of the vagus completely stop the heart, but also even at 120-coil distance the stimulation of the peripheral end of the vagus had a distinct cardio-inhibitory effect. This means that the calcium not only reversed the depressing effect of the magnesium, but increased the irritability of the vagus beyond its original normal threshold. Finally, calcium caused also an immediate rise of the blood pressure, which was greatly reduced by the magnesium. It should be remembered that all these neutralizing effects of the calcium were brought out while the magnesium was still running in at the original rate.

DISCUSSION.

The experiments communicated in this paper, which form only a small fraction of those performed, established that the intravenous infusion of various calcium salts is capable of completely reversing the pronounced inhibitory effects brought on by the various magnesium salts. The respiratory paralysis, the lost lid reflex, the motor paralysis, the lost general reflexes, the general anesthesia, the loss of consciousness, the cardio-in-

ary depression, the lowering of the blood pressure, — all are reversed and restored in a very short time and by a comparatively small quantity of a calcium salt. We do not know of any other instance in biology of such a striking, we may say miraculous, agonistic effect.

We shall not attempt to offer a theory how this effect is accomplished. Any theory constructed from the present facts would be premature and would soon have to be reconstructed. The various hypotheses which one has, and has to have as a guide to direct the further search for facts, had better not see the light of day before their viability is well assured.

For the present we are burdened only with this one hypothesis, that magnesium favors inhibitory processes. All the facts which were brought out so far sustain it. Even the fact brought out by Loeb that *Polyorchis*, which is motionless in a pure sodium chloride solution, starts its rhythmical motions when a magnesium salt is added, means also inhibition and not excitation. The rhythmical swimming movements of this medusa are absent in the pure sodium chloride solution on account of the tonic state of the contractile tissues which is manifested, according to Loeb, in a tonic contraction of the mouth and tentacles. These tonic contractions become relaxed, inhibited by the addition of magnesium.

Another exception to the inhibition hypothesis would seem to offer itself in a fact recorded in this paper, that the cardio-inhibitory effect of the vagus becomes greatly reduced under the influence of strong concentrations of magnesium salts. Here is an inhibitory function in the body which is not intensified by magnesium, but rather depressed. The obstacle, however, is in no way fatal, and some qualification of the hypothesis could be easily made which would then cover also this apparent exception. We prefer, however, to wait and collect more facts before we attempt to give a final form (or even the only provisionally final?) to this hypothesis.

We wish, however, to discuss briefly the nature of the action of calcium. In our present series of experiments calcium was anything but inhibitory; it promptly restored contractility and sensibility. Also, in Loeb's experiments on *Polyorchis*, calcium antagonizes magnesium.

Furthermore, as we have already mentioned, calcium restores the irritability of muscle and nerve lost through the effects of potassium, rubidium, etc., and restores indirect irritability of the

